Abteilung Experimentelle Ökologie der Tiere

Universität Ulm

Influence of introduced predators and natural stressors on escape behavior and endocrine mechanisms in an island species, the Galápagos marine iguana *(Amblyrhynchus cristatus)*

Dissertation

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vorgelegt von

Silke Berger
aus Crailsheim

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"Perhaps this singular piece of apparent stupidity may be accounted for by the circumstance, that this reptile has no enemy whatever on shore."

Charles Darwin

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GENERAL INTRODUCTION

Animal species on islands often experience relaxed selection by predation because they are exposed to fewer types of predators than species on the mainland. Lower diversity is in accordance with the theory on biogeography that predicts fewer species at equilibrium on islands compared with an equal area of the adjacent mainland (MacArthur & Wilson 1967). Island species are often rather tame, i.e. they show very little anti-predator behavior compared to animals on continents (Beauchamp 2004; Magurran 1999; Magurran et al. 1995; Stone et al. 1994). In general, anti-predator behavior is costly in terms of losing time for other activities (Cooper et al. 2004; Martín & López 2003). It also leads to physiological costs as escape responses require energy which can result in a decrease of body condition (Martín & Lopez 1999; Pérez-Tris 2004). Therefore, based on economic considerations (Ydenberg & Dill 1986), costly and no longer functional anti-predator behavior should be lost or reduced when a species is isolated from predators (Blumstein & Daniel 2005).

The low wariness of island species was already observed and described by early researchers (Darwin 1839; Lack 1947). Nevertheless, the range of reaction norms in anti-predator behavior of island species that evolved without serious predation is still little investigated. This, however, is of importance for conservation programs because of the introduction of novel predators on previously predator-free or predator-poor islands by humans worldwide during the last two centuries. Introduced predators are known to have dramatic effects on island populations of birds and reptiles and are known to be the direct cause of severe reduction or even extinction of numerous endemic island species (Cruz & Cruz 1987; Duncan & Blackburn 2004; Iverson 1978; Moors & Atkinson 1984; Pimm 1987; Snell et al. 1984). As an example, an estimated population of 5500 Turks and Caicos rock iguanas *(Cyclura carinata)* on Pine Cay in the Caribbean Caicos Islands was extirpated within five years of the introduction of cats and dogs (Iverson (1978). A further disastrous example is the loss of 10 species of moas, an endemic group of birds in New Zealand, by introduced predators (Bunce et al. 2003). Endemic island species are highly susceptible to extinction because they share traits, such as low escape response or flightlessness that make them especially vulnerable to novel threats (Duncan & Blackburn 2004; McNab 1994).

It is likely that the range of behavioral reaction norms of island species is not wide enough any more to allow for an efficient adjustment of anti-predator behavior to novel threats. Whether an animal is able to adjust its anti-predator behavior may depend on the time course of relaxed selection caused by the laps or minimization of predation pressure. How long a population takes to 'relax' or whether this process is easily reversible still needs to be clarified. In some cases, anti-predator behavior persists for thousand of years after isolation from predators (Coss 1999; Curio 1966), whereas in others it is lost quickly after eliminating predators by humans in the last century (Berger 1998).

Mainland species have evolved a variety of anti-predator behaviors (Magurran et al. 1995; Seghers 1974; Stoks et al. 2003; Van Buskirk 2001) and life-history strategies (Reznick & Endler 1982) to reduce predation risk. They face trade-offs between costly anti-predator responses and other behaviors such as reproductive activities or foraging to optimize the cost-benefit ratio (Lima & Dill 1990; Ydenberg & Dill 1986). As an example, territorial defense can be in conflict with predator avoidance because a territorial resident that is hiding cannot simultaneously defend its territory from conspecific intruders (Díaz-Uriarte 1999). Generally, it may be advantageous for prey to wait with an escape response until the risk of predation is higher than the gain from other behaviors such as feeding and territorial defense (Bonenfant & Kramer 1996; Dill & Houtman 1989; Ydenberg & Dill 1986).

The ability of prey species to detect and avoid predators may depend in part on their evolutionary history, i.e. whether they experienced a long-term co-existence between predator and prey. Consequently, prey species may recognize coevolved and/or sympatric predators earlier as a threat than recently and/or allopatric ones (Catarell & Chanel 1979; Dickman 1992; Müller-Schwarze 1972; Sullivan et al. 1985). The ability for predator recognition and proximate anti-predator responses can vary individually depending on factors such as age, social status and sex (Clutton-Brock & Harvey 1979; Díaz-Uriarte 2001; Greene 1988; Whiting et al. 2003). For example in green iguanas *(Iguana iguana)*, juveniles and females are warier than adults and males probably due to their smaller body size, which makes them more vulnerable to predators (Greene et al. 1978). While age and sex differences in anti-predator behavior and trade-offs between escape response and other requirements are critical for species on the predator-rich mainland, those features might be not that crucial for island species that evolved without strong predation pressure. Therefore, the question remains whether this variation in anti-predator behavior was eliminated in island species by relaxed selection from predators, or whether island species show the same behavioral patterns as known from their mainland relatives.

Anti-predator responses are not only restricted to the behavioral domain, they also contain physiological responses (Blanchard et al. 1998; Eilam et al. 1999; Selye 1946). A major unit of physiological responses after the perception of threats is the hypothalamo-pituitary-adrenal (HPA) axis, which leads after activation to a release of glucocorticoids in the blood stream (Sapolsky et al. 2000). Glucocorticoids, the main stress hormone in vertebrates, can induce behaviors that promotes escape and ultimately survival by leading the animal away from the threat (Astheimer et al. 1995; Breuner et al. 1998). Thus, escape is usually accompanied with an elevation of glucocorticoids in the blood plasma (Astheimer et al. 1992; Orchinik 1998). HPA activation with glucocorticoid release after detection of predation threat occurs in mainland species that are accustomed to local predators (Astheimer et al. 1992; Canoine et al. 2002; Cockrem & Silverin 2002; Eilam et al. 1999; Orchinik 1998; Scheuerlein et al. 2001). However, nothing is known about the sensitivity of the HPA-axis in naïve and "tame" island species because appearance of external "calm" behavior to potential threat is not necessarily associated with the absence of physiological processes in an individual (Hüppop &

Hagen 1990; Wilson et al. 1991). Oystercatchers *(Haematopus ostralegus)* e.g. doubled their heart rate during human approach without any visible signs of escape (Hüppop & Hagen 1990). Generally, environmental perturbations caused by both biotic and abiotic factors may rapidly increase circulating glucocorticoids in wild animals (Greenberg et al. 1984; Wingfield et al. 1994; Wingfield et al. 1992). Increase of glucocorticoids in response to stressors are thought to be adaptive in terms of preparing the animal for immediate life-saving processes (Sapolsky et al. 2000; Wingfield & Ramenofsky 1999). A main function of glucocorticoids is provisioning of energy by mobilization of glucose through gluconeogenesis during strenuous circumstances such as starvation, reproduction or predator attacks (Axelrod & Reisine 1984; Romero & Wikelski 2001; Wingfield 1994).

Furthermore, variations in glucocorticoid levels is a reliable indicator of stress and is frequently used in conservation to detect the impact of various stressors on wildlife (Blanchard et al. 1998; Cockrem 2005; Romero et al. 2004; Wikelski & Cooke 2006; Wingfield et al. 1995). Applying endocrine data for conservation issues is a new discipline, the so-called "conservation endocrinology" (Cockrem et al. 2004). Prolonged exposure to environmental stress may lead to chronically elevated glucocorticoid levels which lead to inhibited growth, reproduction and immune activity (Orchinik 1998; reviewed in Romero et al. 2004). Endocrine studies on wild-living animals provide information on physiological responses of animals to anthropogenic disturbance such as tourists (Fowler 1999; Müllner et al. 2004; Walker et al. 2005), habitat disturbance (Wasser et al. 1997) or pollutants (Fowler 1999; Wikelski et al. 2001). For conservation management decisions, it is particularly important to pay attention to individual differences in the adrenocortical responsiveness because the mean glucocorticoid response of a population does not accurately describe the responses of all individuals (reviewed in Cockrem 2005). Individuals may react differently with regard to adrenocortical responsiveness depending on age, sex and social status (Dunlap & Wingfield 1995; Grassman & Hess 1992; Knapp & Moore 1996). This provides a mechanism for the regulation of behaviors and physiology that are important for particular life-history stages (Wingfield & Kitaysky 2002; Wingfield & Monk 1992). As an example, juvenile green turtles *(Chelonia mydas)* that are assumed to be more vulnerable to predation due to their small body size show higher glucocorticoid concentrations than adults (Jessop & Hamann 2005).

The question arises whether naïve and "tame" island species, which experience low or no predation pressure, adjust their adrenocortical responsiveness when they are exposed to a novel threat. Furthermore, it remains unclear whether glucocorticoid responses vary within a population according to the relative vulnerability of the respective individuals.

THESIS OBJECTIVES, STUDY ANIMAL AND STUDY AREA

The overall perspective of my thesis was to investigate anti-predator responses, both behaviorally and physiologically, in a naïve island species which evolved under relaxed selection from predators in a time-frame of several million years, and its capability to adjust its anti-predator behavior and adreno-cortical stress response to novel threats. I used the marine iguana *(Amblyrhynchus cristatus)* on Galápagos as a system to study the escape and stress physiological response on pristine and on disturbed islands where marine iguanas experience introduced predators such as dogs and cats since the colonization of the islands by humans about 150 years ago.

The age of the islands as we know them today ranges from 500.000 to 5 million years (Rassmann 1997). They are of volcanic origin and were never in contact with the mainland. Furthermore, as far as one can tell, no mammalian predators have ever occurred on these islands. Marine iguanas that are endemic to the Galápagos islands and originated about 5-15 million years ago (Rassmann 1997), evolved under relaxed selection from predators because the only native predators are the Galápagos hawk *(Buteo galapagoensis)* and some herons (e.g. *Nicticorax nicticorax, Butorides sundevalli*), which mainly prey on smaller individuals. Marine iguanas are widespread on most of the islands of the archipelago and live in huge aggregations at the rocky shores. They developed extensive basking behavior where they lie exposed on the black lava rocks to heat up their body temperature. The marine iguanas exhibit only little escape behavior when approached by humans. This "tame" behavior has become a serious problem since the introduction of predators. The growing numbers of dogs and cats result in mortality rates of up to 27 % in marine iguanas (Kruuk & Snell 1981) or even more in some populations (Rödl et al. accepted). However, the new predators have not yet reached all islands of the archipelago. This scenario offers a large-scale experimental setting where some marine iguana populations have been exposed to novel predators in recent times and other populations still live on islands without dogs or cats.

THESIS TOPICS AND SUMMARY OF CHAPTERS

My thesis is divided into five chapters aimed at investigating the anti-predator responses of marine iguanas at sites with and without introduced predators. The main topics encompass:

1. Individual differences in anti-predator behavior according to life-history stages and trade-offs between escape response and other demands in marine iguanas at an undisturbed site *(Chapter 1)*.
2. Sensitivity of the HPA-system to a potential biotic stressor, the reproductive period, in male marine iguanas at an undisturbed site *(Chapter 2 and 3)*.
3. Correlation between the HPA-system and immune activity *(Chapter 2)*.

4. Influence of a novel threat, the introduced dogs and cats, on anti-predator behavior and adrenocortical responsiveness of marine iguanas *(Chapter 4)*.

5. Influence of an acute stressor such as experimental chasing on the HPA-system of marine iguanas at sites with and without introduced predators *(Chapter 5)*.

6. Influence of negative experiences on anti-predator behavior and/or adrenocortical stress response of marine iguanas at sites with and without introduced predators *(Chapter 1, 4 and 5)*.

In **Chapter 1**, the objective was to determine whether marine iguanas balance the risks and costs of escape according to the optimal escape theory and to assess individual differences in anti-predator behavior in relation to age, sex, and social status (Ydenberg & Dill 1986). For instance, theory predicts that territorial males face a trade-off between predator avoidance and territorial defense (Cooper Jr. 1999; Cooper 1998; Díaz-Uriarte 2001), or that individuals with lower body temperature should increase anti-predator behavior to compensate for reduced sprint speed (Bennett 1980; Rand 1964; Smith 1997).

To investigate anti-predator behavior, I measured flight initiation distance (FID) to a simulated predator approach consisting of an experimenter walking slowly up to marine iguanas of different age, sex and male reproductive phenotype, and to animals with different body temperature throughout the day to detect possible correlations with FIDs. Furthermore, I investigated whether marine iguanas are capable to adjust their anti-predator response by negative experiences such as previous capture and handling.

The results indicate that naïve marine iguanas exhibited only minor adjustments in anti-predator behavior. I found significant differences in FID between hatchlings, juveniles and adults. Juveniles had the highest FIDs of all age classes. However, the adjustment of juveniles was negligible (only 0.6 m in average) and probably only sufficient for island scenarios with low predation threat. Sex differences in escape behavior varied seasonally, i.e. females showed higher FIDs than males during the nesting period when they dig nest holes at sandy areas, thus becoming easy prey to the native Galápagos hawk *(Buteo galapagoensis)*. In contrast to various mainland species, I did not find lower FIDs in territorial active males compared to non-territorial satellites and bachelors, what suggests that territorials did not trade off between escape response and reproductive behavior. Furthermore, marine iguanas did not compensate with larger FIDs when slowed down by low body temperature. Although male marine iguanas learned to adjust FID after just one negative experience, this accounted only for minor differences and could only be elicited during the reproductive period. During the non-reproductive period marine iguanas did not show any learning after negative experience.

In addition to the escape behavior of naïve marine iguanas, I examined their adrenocortical stress response under the influence of a natural stressor, the reproductive period. In **Chapter 2** I compared three male reproductive phenotypes, territorials, satellites and bachelors. Territorial males occupy and

defend territories to attract females. Satellites do not show territorial behavior but roam around to force copulations with females, whereas bachelors are reproductively inactive and gather with other bachelor males without any signs of aggressive interactions (Carpenter 1966; Eibl-Eibesfeldt 1966). Territorials are the most ornamented and reproductively active phenotype compared to bachelors and satellites.

Since glucocorticoids metabolize energy reserves for activities (McEwen & Wingfield 2003; Romero & Romero 2002; Wingfield et al. 1998), I measured corticosterone (CORT), the main glucocorticoid in reptiles, in the blood plasma of males with distinct reproductive phenotypes to examine whether the amount of reproductive activity is correlated with CORT concentrations. Furthermore, I compared the plasma concentrations of testosterone (T), the main sex hormone responsible for reproductive behavior and ornamentation, between phenotypes. I also compared the cell-mediated immune response between reproductive phenotypes to test the "immunocompetence handicap hypothesis" proposed by Folstadt and Karter (1992). The hypothesis suggests a trade-off between T-related secondary sexual characters and immune activity because both reactions are costly to the respective individuals (Demas et al. 1997; Gustafsson et al. 1995; Hanssen et al. 2004; Lochmiller & Deerenberg 2000; Martin et al. 2003; Raberg et al. 2000; Sheldon & Verhulst 1996). I proposed that territorials as the most ornamented phenotype should have the highest T concentrations and concomitantly the lowest immune response.

Contrary to expectation, I found that the reproductive phenotypes had indistinguishable T concentrations. However, CORT concentrations and body condition differed significantly between phenotypes. Territorial males, the males with the most elaborate behaviors, had higher CORT concentrations and lower body condition and immune responses than satellites or bachelors. The data demonstrate that CORT can induce immunosuppression. However, they do not support the immunocompetence handicap hypothesis in its narrow sense which proposes a negative correlation between T concentrations and immune activity.

In **Chapter 3** I investigated the adrenocortical stress response, T concentrations and body condition associated with reproductive activities in male marine iguanas over the course of the reproductive period. Male marine iguanas are capable of rapid switches between reproductive phenotypes (bachelor = non-reproductive, satellite = intermediate reproductive activity, territorial = highest reproductive activity) within a season.

I studied males during one reproductive season and reported endocrine correlates of rapid phenotype transitions. I compared plasma concentrations of T and CORT and body condition between males that switched reproductive phenotype (1) from a less to a more active phenotype, (2) from a more to a less active phenotype, or (3) males that maintained the phenotype during the reproductive season at times of territorial establishment (before potential phenotype switch) and mating (after phenotype switch). I hypothesized that CORT and/or T concentrations should increase in males that

switched from a less to a more active phenotype and should decrease when males switch from a more to a less active phenotype because CORT and T are known to be related with aggression/activity in many vertebrate species (Wikelski et al. 2004, Wingfield & Marler 1987, reviewed in Wingfield et al. 1990). In contrast, bachelor males, the non-reproductive group, should show low hormone concentrations throughout the course of reproduction.

Contrary to expectation, I found that CORT concentrations increased while body condition indices decreased in all males independently from phenotype and type of switch. These results point to abiotic environmental effects rather than an impact of reproductive behavior per se. T remained at its initial high concentrations during reproduction in males that switched from a less to a more active phenotype, whereas T concentrations decreased in males that switched vice versa or maintained phenotype.

While the studies in Chapter 1 to 3 have been conducted on the pristine island Santa Fé, where no anthropogenic disturbance occurs, in **Chapter 4** I compared phenotypic plasticity in anti-predator behavior and adrenocortical stress response of marine iguanas between disturbed and undisturbed sites, i.e. sites with and without introduced predators, namely dogs and cats.

The aim of this chapter was to test whether the marine iguanas' behavior and stress physiology change after the introduction of new predators. I also tested for individual differences according to age and sex in escape and adrenocortical response. I measured FIDs to simulated predator approach and CORT concentrations (baseline and stress-induced concentrations) in marine iguanas of different age and sex at sites with and without new predation threat. I hypothesized that FID and CORT concentrations (baseline and/or response concentrations) increase in populations exposed to cats and dogs to cope with the novel predators. Furthermore, I expected age and sex differences associated with the relative vulnerability to predation.

Indeed, the results demonstrate that local populations of marine iguanas reacted differentially to simulated predator approaches. FIDs were larger on sites with predation than on sites without predation. Furthermore, the occurrence of new predators was associated with increased CORT response concentrations in marine iguanas. Age also turned out to be a strong predictor of variation in FID, CORT baseline and response concentrations. Juveniles, which are probably more threatened by predators due to their small body size, showed larger FIDs and higher CORT baseline and response concentrations than adults.

In **Chapter 5** we investigated the physiological control of tame behavior in marine iguanas and its flexibility in the face of predator introduction (Blazquez et al. 1997; Blumstein & Daniel 2005). We experimentally tested the behavioral and adrenocortical stress response of marine iguanas to a novel threat and replicated our experiments at three sites with differing predation pressure caused by introduced dogs and cats.

We measured FIDs of marine iguanas from three islands with individuals (a) living without predators (b) living with intermediate predation and (c) living with acute, heavy predation from introduced dogs and cats. Some study animals were naïve, while we exposed others to negative experiences by capture and handling prior the simulated predator approach. Furthermore, we measured the adrenocortical stress response to acute disturbance in naïve and experienced individuals. To cause acute disturbance, a human experimenter pursued a focal animal for 15 minutes without physical contact ("chasing"). After 15 minutes of chasing, the animal was captured and a blood sample was taken to measure the CORT concentration.

We found that marine iguanas living without or with intermediate predation, did not show a physiological stress response to experimental chasing. In contrast, marine iguanas on San Cristobal, a site with heavy and acute predation threat, showed a significant CORT increase to experimental chasing by a human. Furthermore, marine iguanas quickly restored their adrenocortical stress response after capture and handling experience, provided they lived at sites with introduced predators. FID increased with negative experience, but remained too low to efficiently escape introduced predators, what is indicated by the high mortality rate on San Cristobal.

GENERAL CONCLUSIONS

Chapter 1 revealed that marine iguanas, living on islands with low predation pressure caused by only few native predators, lack several traits associated with predator avoidance characteristic for mainland species. The behavioral adjustments to native predation threat according to life-history stages (e.g. juveniles) are minor and only sufficient in an island scenario with relaxed selection from predators. On the mainland, however, those adjustments in FID would not be sufficient to escape predators. This finding is supported by a recapture rate of 100 % in marine iguanas that had been previously captured and handled (Berger and Rödl unpublished data). Overall, low anti-predator response is seen as one of the adaptations of species living in predator-poor environments, mostly to avoid costs if escape is not functional. As a consequence, island iguanas become highly vulnerable which increases mortality rate through new predators that have been introduced.

While anti-predator behavior is reduced in marine iguanas, the adrenocortical stress response works efficiently and is sensitive to natural stressors such as the reproductive period as it is known from numerous animal species (Sapolsky 1987; Wingfield 1984; Wingfield & Kitaysky 2002). Glucocorticoids mobilize energy for strenuous activities, therefore, the variability in CORT concentrations between male marine iguanas of different reproductive phenotypes as presented in **Chapter 2** may be explained by the different amounts of energy a phenotype requires. Territorial males exhibited the highest CORT concentrations probably because the costs needed to acquire and maintain territorial status are higher than being a satellite or bachelor (Goymann & Wingfield 2004). I conclude that ener-

getic costs and, concomitantly, concentrations of plasma CORT are high in territorials because they need to defend their territories which can only be maintained with constant physical aggression towards rivals. The data also showed immunosuppression by CORT, providing evidence for a trade-off between reproductive activity and immune responses. Finally, I propose that CORT has an important integrative role in modulating marine iguana's behavior and physiology during strenuous times.

I also found that the HPA-system of reproducing marine iguanas may be strongly influenced by environmental changes within a season. In **Chapter 3** all male marine iguanas, independent of phenotype or switch thereof, showed increasing CORT levels and concomitantly decreasing body condition over the course of the reproductive season. This indicates that environmental factors, such as climatic changes or food availability, have a significant influence on CORT concentrations rather than reproductive activity per se. This corresponds well to the role of CORT that helps to metabolize energy resources during food scarcity (Dunlap 1995; Romero & Wikelski 2001). During the reproductive period, air and sea surface temperature increase which leads to a decrease in algae growth, the main food of this sea going reptile (Rubenstein & Wikelski 2003; Wikelski et al. 1997). Overall, the changing environment is probably the main reason for the rapid phenotype transition of some male marine iguanas. This means that the decision whether or not to reproduce is extended far into the reproductive season. This may be an adaptation to the unpredictable climate of the Galápagos Islands.

Plasma T concentrations of males did not show any consistent pattern in association with different phenotypes. T appeared to capacitate all males to participate in reproduction because T levels were highest during the early phase of territorial establishment in all males independent on their initial phenotype. T concentrations remained high in males that switched from a less to a more active phenotype, probably to allow phenotype transition and thereby territorial aggression. However, in all other males, including males that maintained the territorial phenotype, T concentrations decreased over the course of the reproductive season, although territorial aggression usually does not decrease during this period. Altogether, rapid phenotype transition within a season is probably supported by T and helps to maximize lifetime reproductive success in a variable and unpredictable environment like the Galápagos archipelago.

In addition to the natural variation of the environment, marine iguanas on Galápagos face ongoing environmental changes by human impact, i.e. growing numbers of introduced predators such as dogs and cats. Due to this new predation pressure, it is important for conservation programs to know the range of reaction norms in the anti-predator responses of naïve island species to develop meaningful management plans. The results of **Chapter 4** supply evidence that naïve island species show behavioral and physiological plasticity, which is adaptive and associated with actual predation pressure. Marine iguanas at sites with introduced predators exhibited significantly higher FIDs and CORT concentrations than individuals at pristine sites. However, the behavioral adjustments reached only a certain degree and are not sufficient to deal with the novel predators. This may drive many island species to extinction.

An important question is, whether the low wariness of a naïve island species is accompanied by the failure to activate the HPA-system, suggesting deficient predator recognition. Indeed, as shown in **Chapter 5**, the adrenocortical responsiveness to an acute stressor ("chasing"), which is known from mainland species, was missing in naïve marine iguanas. Conversely, a one-time experience with a predation attempt (capture) was enough to increase the escape response in marine iguanas and, in conjunction with the presence of the predator, activated an adrenocortical response. To conclude, living among predators seems to prime the HPA-system, which then allows a rapid change from being insensitive to being responsive to a novel stressor (the chasing experimenter). Since the HPA-system is important for appropriate reactions towards environmental stressors such as food scarcity by El Niño events (Romero & Wikelski 2001), marine iguanas appear to retain the adrenocortical function even through long evolutionary periods of time with relaxed selection from predators. However, the increase of FIDs after a one-time negative experience was small in marine iguanas. Thus, we suggest high phenotypic plasticity of the adrenocortical response to novel stressors but little plasticity in escape behavior.

Overall, I propose that the "tameness" of marine iguanas is possibly associated with a lack of the adrenocortical stress response to potential threats as a result of the estimated 5 – 15 million years of predator release. Although both, anti-predator behavior and physiological response can be adjusted to novel threats, the range of reaction norms in escape behavior is currently too narrow for marine iguanas to adjust fast enough to unknown threats in order to minimize predation by introduced predators. In contrast to the external behavior, the physiological stress response showed high phenotypic plasticity to novel predators, i.e. it increased in marine iguanas at sites with introduced dogs and cats and after one-time negative experience of handling and capture. However, the physiological adjustments of marine iguanas are not associated with a sufficiently elevated wariness to avoid predation leading to the high mortality rates by introduced predators which have driven populations already near extinction.

The lack of wariness of naïve island species towards novel predators is a general phenomenon worldwide and one of the main reasons for the decreasing number of island species. To better predict the impact of novel predators and to protect endangered island species requires further studies on the generality of the presumed limited range of reaction norms in predator-naïve organisms. This will improve our knowledge on the interactions between wariness and its physiological control mechanism and will help to plan conservation programs to protect endangered island species. For instance, the results may provide useful information for re-introduction programs training naïve animals to recognize and cope with novel threats.

PUBLICATIONS FROM THIS THESIS AND CONTRIBUTIONS FROM CO-AUTHORS

My thesis contains five chapters, each representing a separate publication. Chapter 2 has already been published in Hormones and Behavior. Chapter 5, where I am the second author, is accepted in Proceedings of the Royal Society of London – Series B: Biological Sciences. Chapter 1, 3, and 4 are prepared for submission.

Chapter 1: Berger, S., Wikelski, M., Kalko, E. K. V. & Rödl, T. *prepared for submission*. The influence of life history stages on flight initiation distance in naïve Galápagos marine iguanas.

Chapter 2: Berger, S., Martin II, L. B., Wikelski, M., Romero, L. M., Kalko, E. K. V., Vitousek, M. N. & Rödl, T. 2005. Corticosterone suppresses immune activity in territorial Galápagos marine iguanas during reproduction. *Hormones and Behavior* 47, 419-429.

Chapter 3: Berger, S., Wikelski, M., Kalko, E. K. V. & Rödl, T. *prepared for submission*. Rapid phenotype transition within a reproductive season in male marine iguanas – a matter of hormones and the environment?

Chapter 4: Berger, S., Wikelski, M., Romero, L. M., Kalko, E. K. V. & Rödl, T. *prepared for submission.* Behavioral and physiological adjustments to new predators in an endemic island species, the Galápagos marine iguana.

Chapter 5: Rödl, T., Berger, S., Romero, L. M. & Wikelski, M. 2006. Tameness and stress physiology in a predator-naïve island species confronted with novel predation threat. *Proceedings of the Royal Society of London – Series B: Biological Sciences* online.

My contribution:
Idea, experimental design, data collection, lab work, analysis and writing

Contributions from co-authors:
Prof. Elisabeth K. V. Kalko: Comments to manuscripts, discussions on experimental design
Prof. Martin Wikelski: Lab space and consumables, research permit, comments to manuscripts, discussions on experimental design
Dr. Thomas Rödl: Idea, experimental design, data collection, lab work, analysis and writing
Prof. L. Michael Romero: Comments to manuscripts, lab space and consumables, lab advice
Dr. Lynn B. Martin II: Comments to manuscript, discussions on experimental design
Maren N. Vitousek: Field assistance

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Zusammenfassung

ALLGEMEINE EINLEITUNG

Inseltiere sind häufig nur geringem Prädationsdruck ausgesetzt und verhalten sich deshalb meist sehr „zahm", d.h. sie zeigen kaum Fluchtreaktion bei menschlicher Annäherung. Da Fluchtverhalten im allgemeinen Kosten verursacht, beispielsweise in Form von Energieverbrauch, entgangener Reproduktionsaktivität oder Nahrungsaufnahme, ist es für Inselarten aus ökonomischen Gründen sinnvoll, nicht unmittelbar benötigtes Verhalten zu reduzieren oder abzulegen.

Die Zahmheit von auf Inseln lebenden Vertebraten wurde bereits von zahlreichen Wissenschaftlern beschrieben. Jedoch sind die Spannbreite der Reaktionsnorm und die Anpassungsfähigkeit von Fluchtverhalten bei Inselarten noch unzureichend untersucht. Im Bereich des Umweltschutzes erlangt dies enorme Wichtigkeit, da Inselarten aufgrund ihres fehlenden Fluchtverhaltens durch den Menschen und durch von Menschen eingeführte Prädatoren zunehmend bedroht werden. Eingeführte Hunde und Katzen beispielsweise haben dramatische Auswirkungen auf Inseltierwelten und sind oftmals verantwortlich für die Abnahme oder das Aussterben von Inselarten. So wurde eine Population von 5500 endemischen Turks- und Caicos-Leguanen auf den karibischen Caicos Inseln innerhalb von fünf Jahren durch Hunde und Katzen ausgelöscht. Demnach ist die Spannbreite der Reaktionsnorm bei den betroffenen Inselarten vermutlich nicht groß genug, um effizient auf neue Bedrohungen zu reagieren. Ob eine Inselart in der Lage ist, ihr Fluchtverhalten an neue Gefahren anzupassen, hängt wahrscheinlich maßgeblich von der Zeitdauer der Prädatorabwesenheit ab. Wie lange eine Population benötigt, um ein bestimmtes Verhalten abzulegen und ob dieser Prozeß reversibel ist, ist jedoch noch nicht ausreichend geklärt. In manchen Fällen bleibt das Anti-Prädatorverhalten trotz mehrtausendjähriger Abwesenheit von Raubfeinden bestehen, wie beispielsweise das Anti-Schlangenverhalten bei Kalifornischen Erdhörnchen. In anderen Fällen dagegen, z.B. bei Elchen, ging die Sensibilität gegen Bären und Wölfe schon bereits nach 100 Jahren verloren.

Tiere am Festland, die ständigem Prädationsdruck ausgesetzt sind, haben zahlreiche Strategien zur Prädationsvermeidung entwickelt. Dabei müssen Festlandarten Kompromisse nach Kosten-Nutzen-Prinzipien eingehen, d.h. sie wägen ab, zu welchem Zeitpunkt es ökonomisch sinnvoll ist zu fliehen und wann es von Vorteil ist, sich anderen Aktivitäten wie Reproduktion und Nahrungsaufnahme zu widmen. Das bedeutet, daß ein Tier erst fliehen wird, wenn die Kosten, nicht auf den Prädator zu reagieren, höher sind als der Nutzen des alternativen Verhaltens. Nicht alle Individuen innerhalb einer Population verhalten sich hierbei gleich. Häufig gibt es deutliche individuelle Unterschiede bezüglich Aufmerksamkeit und Fluchtinitialisierung im Zusammenhang mit Alter, Geschlecht und sozialem Status. Juvenile und Weibchen sind oftmals aufgrund ihrer kleineren Körpergröße und der damit verbundenen höheren Prädationsgefahr aufmerksamer und scheuer als Adulte und Männchen. Auch individuelle Erfahrungen beeinflussen die Prädationsvermeidung. Individuelle Unterschiede und die

Abwägung zwischen Prädationsvermeidung und anderen Verhaltensweisen sind besonders für Arten wichtig, die starkem Prädationsdruck ausgesetzt sind, um ihre Fitneß zu optimieren. Es stellt sich deshalb die Frage, ob Inselarten trotz des schwachen oder zum Teil auch vollkommen fehlenden Prädationsdrucks und ihres geringen Fluchtverhaltens ähnliche Kompromisse und Muster in der Prädationsvermeidung zeigen, wie ihre Verwandten am Festland.

Die Reaktionen von Beutetieren auf Prädatoren sind jedoch nicht nur auf Fluchtverhalten beschränkt, sondern gehen mit physiologischen Prozessen einher. Eine wichtige Einheit hierfür ist die Hypothalamus-Hypophyse-Nebennierenrinde-Achse (hypothalamo-pituitary-adrenal, HPA). Bei Aktivierung der HPA-Achse durch Wahrnehmung von Gefahr kommt es zu einer Ausschüttung von Glukokortikoiden (GC), dem Hauptstreßhormon bei Vertebraten, das die Fluchtreaktion unterstützt. GC-Ausschüttung bei Annäherung eines Prädators wurde bei verschiedenen Festlandarten gezeigt, z.B. bei Europäischen Schwarzkehlchen *(Saxicola torquata rubicola)*, die einem Waldkauz *(Strix aluco)* ausgesetzt wurden. Ob Inseltiere trotz ihres augenscheinlich „zahmen" Verhaltens bei potentieller Bedrohung ebenfalls physiologische Reaktionen zeigen, ist noch nicht bekannt.

Eine wichtige Funktion von GCs ist die Mobilisierung von Energiereserven. Deshalb gilt der Anstieg von GCs im Blutplasma ausgelöst durch Stressoren, wie beispielsweise bei einem Prädatorangriff, Nahrungsknappheit oder während aggressiver Interaktionen, als Anpassung an Zeiten erhöhten Energiebedarfs. Veränderungen in den GC-Konzentrationen stellen außerdem einen wichtigen Indikator für Streß dar und werden häufig für Untersuchungen im Naturschutzbereich über den Einfluß von Störungen auf Wildtiere herangezogen. Langanhaltend erhöhte GC-Konzentrationen bedeuten, daß ein Tier durch einen Stressor, wie beispielsweise Tourismus, Umweltverschmutzung oder Habitatzerstörung, chronisch gestreßt sein kann. Der Einfluß von Störfaktoren auf Wildtiere kann sich dabei individuell je nach Alter, Geschlecht und sozialem Status unterscheiden. Ein Beispiel ist die Altersabhängigkeit der GC-Konzentrationen, d.h. bei einigen Tierarten wie zum Beispiel bei der Suppenschildkröte *(Chelonia mydas)* konnte gezeigt werden, daß Juvenile aufgrund ihrer erhöhten Prädationsgefahr höhere GC-Konzentrationen aufweisen als Adulte. Deshalb ist es für den wissenschaftlichen Naturschutz wichtig, auch diese Faktoren in die Untersuchung mit einzubeziehen. Es gilt daher zu klären, ob Inselarten, die unter geringem Prädationsdruck evolviert sind, ihre GC-Konzentrationen durch die Anwesenheit von eingeführten Prädatoren erhöhen und ob sich die GC-Konzentrationen je nach Ausmaß der Prädationsgefahr individuell unterscheiden.

Für die Untersuchung des Fluchtverhaltens und der physiologischen Streßreaktion bei Inseltieren wählte ich die Galápagos Meerechse *(Amblyrhynchus cristatus)* als System. Sie lebt bereits seit bis zu 15 Millionen Jahren auf den Galápagos Inseln. Die einzigen heimischen Prädatoren an Land sind der Galápagos Bussard *(Buteo galapagoensis)* und einige Reiherarten, die jedoch hauptsächlich Jungtiere und kleinere Individuen erbeuten. Meerechsen zeigen daher bei menschlicher Annäherung kaum Fluchtverhalten und verbringen die meiste Zeit des Tages sonnenbadend auf Lavafelsen. Aufgrund

dieses sehr offenkundigen und zahmen Verhaltens sind Meerechsen durch eingeführte Prädatoren wie Hunde und Katzen ernsthaft bedroht. Direkt betroffen sind jedoch bisher nur Meerechsenpopulationen, die auf einer der fünf bewohnten Inseln des Archipels leben, da es auf den restlichen Inseln noch keine Hunde und Katzen gibt. Dies ermöglichte mir vergleichende Untersuchungen zur Prädationsvermeidung und Streßphysiologie von Meerechsen an Orten mit und ohne Störung und Studien zur Anpassungsfähigkeit an neue Gefahren einer durch geringe Fluchtreaktionen gekennzeichneten Inselart. Zunächst sollten mögliche physiologische Mechanismen der Zahmheit geklärt werden. Außerdem untersuchte ich, ob eventuelle Verhaltensanpassungen bei Meerechsen mit physiologischen Anpassungen einhergehen.

Meine Doktorarbeit ist in fünf Kapitel unterteilt und befaßt sich mit folgenden Themen:

1. Individuelle Unterschiede in der Prädationsvermeidung und Kompromisse zwischen Fluchtverhalten und anderen Verhaltensweisen (z.B. Reproduktionsverhalten) bei Meerechsen auf einer ungestörten Insel *(Kapitel 1)*.

2. Sensibilität des HPA-Systems gegenüber eines natürlichen Stressors (Reproduktion) bei Meerechsenmännchen auf einer ungestörten Insel *(Kapitel 2 und 3)*.

3. Zusammenhang zwischen dem HPA-System und der Immunaktivität *(Kapitel 2)*.

4. Einfluß einer neuen Bedrohung durch eingeführte Hunde und Katzen auf das Fluchtverhalten und der adrenalen Streßreaktion bei Meerechsen *(Kapitel 4)*.

5. Einfluß von akuter, experimentell ausgelöster Störung auf das HPA-System bei Meerechsen an Orten mit und ohne eingeführte Prädatoren *(Kapitel 5)*.

ZUSAMMENFASSUNG DER KAPITEL

Ziel von **Kapitel 1** war es zu klären, ob Meerechsen bei potentieller Bedrohung (z.B. menschliche Annäherung) trotz ihres geringen Fluchtverhaltens Kosten und Nutzen einer Flucht nach ökonomischen Prinzipien abwägen und sich dementsprechend verhalten. Ein Beispiel sind territoriale Männchen, die im Konflikt zwischen Flucht- und Reproduktionsverhalten stehen. Demnach können bei Verlassen des Territoriums durch den möglichen Verlust von Territorium und Kopulationschancen erhebliche Kosten entstehen. Ein zweites Beispiel sind poikilotherme Tiere, die ihre durch niedrige Körpertemperaturen reduzierte Laufgeschwindigkeit mit erhöhter Aufmerksamkeit kompensieren müssen, um einem Prädator erfolgreich zu entkommen. Außerdem untersuchte ich individuelle Unterschiede im Fluchtverhalten bezüglich Alter, Geschlecht und sozialem Status. Dazu wählte ich Meerechsenpopulationen, die auf einer ungestörten Insel ohne eingeführte Prädatoren leben und nur einem sehr geringen Prädationsdruck durch den heimischen Galápagos Bussard ausgesetzt sind.

Zur Untersuchung des Fluchtverhaltens bestimmte ich Fluchtdistanzen (FIDs) bei einer simulierten Prädatorannäherung (Annäherung durch Experimentator) von Meerechsen bei Tieren (1) unterschiedlicher Altersgruppen (Erstjährige, Juvenile und Adulte), (2) unterschiedlichen Geschlechts, (3) mit unterschiedlichen Körpertemperaturen und (4) von Männchen mit unterschiedlichem reproduktiven Phänotyp (Bachelor, Satellit oder Territorialer). Außerdem untersuchte ich, ob Meerechsen in der Lage sind, ihr Fluchtverhalten durch vorherige negative Erfahrung (z.B. Fang) anzupassen.

Juvenile Meerechsen zeigten längere FIDs im Vergleich zu Adulten, vermutlich weil sie dem Prädationsdruck durch Bussarde aufgrund ihrer kleineren Körpergröße stärker ausgesetzt sind. Die Erhöhung der FIDs betrug jedoch im Durchschnitt nur 0,7 m. Weibchen hatten längere FIDs als Männchen, aber nur in der Zeit von Nestbau und Eiablage. Auch dies ist vermutlich eine Anpassung an einen etwas erhöhten Prädationsdruck durch die Bussarde, da Weibchen an den Nestplätzen stärker exponiert sind, als an der Felsküste. Im Gegensatz dazu zeigten adulte Männchen unterschiedlicher reproduktiven Phänotypen, die sich deutlich in ihrer reproduktiven Aktivität und Aggressivität unterscheiden, keine Unterschiede in ihrem Fluchtverhalten. Den ökonomischen Prinzipien entsprechend hätte ich bei den reproduktiv aktiveren Territorialen kürzere FIDs als bei den Satelliten oder nicht reproduktiven Bachelor Männchen erwartet. Eine Kompensierung der geringeren Laufgeschwindigkeit bei Meerechsen mit niedriger Körpertemperatur mit längeren FIDs lag ebenfalls nicht vor. Männchen waren jedoch in der Lage, ihr Fluchtverhalten nach negativer Erfahrung durch Fang zu erhöhen. Dies geschah nur während der Reproduktionszeit. Außerhalb der Reproduktionszeit konnte ich keinen Lerneffekt beobachten.

Die Ergebnisse aus Kapitel 1 zeigen, daß die individuellen Unterschiede und Anpassungen bei erhöhtem Prädationsrisiko sehr gering waren und keine Kosten-Nutzen Abwägungen bezüglich der Entscheidung Flucht oder Verbleiben vorlagen, wie man sie von Tieren kennt, die unter starkem Prädationsdruck stehen. Vermutlich sind diese Anpassungen jedoch ausreichend, um mit dem geringen Prädationsdruck auf der Insel zurecht zu kommen. Außerdem werden dadurch Kosten durch unnötige Flucht verhindert, was vermutlich wiederum eine Anpassung an eine raubfeindarme Umwelt darstellt. Die Konsequenz daraus ist jedoch, daß Meerechsen, wie auch andere Inselarten mit gering oder gar nicht ausgeprägter Fluchtreaktion, sehr anfällig gegen eingeführte Prädatoren sind, da sie aufgrund ihrer geringen Anpassungsfähigkeit auf neue Bedrohungen nicht adäquat reagieren können.

Anti-Prädatorreaktionen sind nicht nur auf Verhalten beschränkt, sondern gehen meist mit physiologischen Reaktionen einher. Deshalb untersuchte ich neben dem Fluchtverhalten bei den Meeresechsen zusätzlich die Sensitivität des HPA-Systems. Hierfür testete ich zuerst die Reaktion der HPA-Achse auf einen natürlichen Stressor, in diesem Fall Reproduktion. In **Kapitel 2** verglich ich Männchen mit unterschiedlichen reproduktiven Phänotypen (Bachelor, Satelliten, Territoriale), die sich in ihrem Reproduktionsverhalten (Aktivität, Aggressivität) deutlich unterscheiden. Territoriale Männchen verteidigen ihre Reviere, zeigen sehr auffallende, sexuelle Merkmale, insbesondere eine deutlich grüne

Färbung an Kopf und Rücken sowie einen geschwollenen Kamm und Nacken und haben die höchste reproduktive Aktivität. Satelliten verteidigen keine Territorien, sondern streifen umher und versuchen Kopulationen mit Weibchen zu erzwingen. Bachelor sind nichtreproduktive Männchen. Sie zeigen keine äußerlich sichtbaren typischen sexuellen Merkmale und verhalten sich nicht aggressiv. Da GCs für die Energiemobilisierung bei erhöhter Aktivität verantwortlich sind, untersuchte ich die Konzentration von Kortikosteron (CORT), dem Hauptstreßhormon bei Reptilien, im Blutplasma. Außerdem verglich ich die Testosteronkonzentration (T), Immunreaktion und die körperliche Konstitution zwischen den verschiedenen Phänotypen. T, das für die Entwicklung der sexuellen Merkmale und für das reproduktive Verhalten verantwortlich ist, kann gleichzeitig immunsuppressive Wirkungen haben. Dieser Zusammenhang ist in der „Immunocompetence Handicap Hypothese" von Folstadt und Karter (1992) beschrieben. Die Hypothese besagt, daß ein trade-off zwischen T induzierten sexuellen Merkmalen und der Immunaktivität vorliegt, da beides energetisch sehr aufwendig ist. Ich nahm daher an, daß territoriale Meerechsen als sexuell auffälligste Gruppe die höchsten T Konzentrationen bei gleichzeitig geringer Immunreaktion aufzeigen sollten.

Während sich CORT Konzentrationen zwischen den Phänotypen unterschieden, traf dies entgegen der Annahme nicht auf T Konzentrationen zu. Territoriale Männchen hatten die höchsten CORT Konzentrationen und gleichzeitig die geringsten Immunreaktionen. Die Ergebnisse zeigen daher, daß die Immunaktivität bei reproduktiven Meerechsen eher von CORT beeinflußt war als von T, und somit die Immunocompetence Handicap Hypothese in diesem Fall nicht unterstützt werden konnte.

Insgesamt schließe ich aus meinen Ergebnissen, daß das HPA-System der Meerechsen sensibel auf natürliche Stressoren, wie Reproduktion, reagiert und CORT eine wichtige Rolle für das Reproduktionsverhalten von Meerechsenmännchen spielt. Demnach mobilisiert CORT bei territorialen Männchen die nötige Energie, um Territorien zu etablieren und zu verteidigen.

Meerechsenmännchen sind grundsätzlich in der Lage, ihren reproduktiven Phänotyp innerhalb einer Reproduktionsperiode zu wechseln. Über den Mechanismus und die Funktionalität dieses Phänomens ist bisher noch wenig bekannt, es wird jedoch angenommen, daß der Phänotypwechsel eine Anpassung an veränderte Umweltbedingungen darstellt. In **Kapitel 3** untersuchte ich deshalb den Zusammenhang zwischen CORT und/oder T und reproduktiver Aktivität von Meerechsenmännchen im Laufe der Reproduktionsperiode, das heißt zu Beginn und zum Höhepunkt der Reproduktion. Zusätzlich bestimmte ich die Veränderung der körperlichen Konstitution über die Reproduktionszeit hinweg.

Ich verglich drei verschiedene Männchengruppen: Männchen, die (1) von einem weniger aktiven zu einem deutlich aktiveren Phänotyp wechselten, (2) von einem aktiveren zu einem weniger aktiven Phänotyp wechselten oder (3) ihren Phänotyp beibehielten. Ich erwartete, daß Männchen, die von einem weniger aktiven zu einem aktiveren Phänotyp wechselten, gleichzeitig ihre T und/oder CORT Konzentrationen erhöhen. Männchen dagegen, die in die entgegengesetzte Richtung wechselten, sollten abnehmende Hormonkonzentrationen zeigen. Als Kontrolle galten Männchen, die über die gesam-

te Reproduktionszeit ihren Bachelor- oder Territorialenstatus behielten. Bachelor Männchen sollten als nichtreproduktive Gruppe gleichbleibend niedrige Hormonkonzentrationen über die gesamte Reproduktionszeit hinweg zeigen, während ich bei Territorialen anhaltend hohe Konzentrationen erwartete.

Entgegen meiner Annahmen fand ich keine Unterschiede in den CORT Profilen zwischen den Männchengruppen. Stattdessen zeigten alle Männchen im Laufe der Reproduktionsperiode ansteigende CORT Werte. Dieses Ergebnis läßt darauf schließen, daß abiotische Faktoren wahrscheinlich einen größeren Einfluß auf die CORT Werte hatten als das Reproduktionsverhalten an sich. T Konzentrationen spiegelten dagegen vermutlich vorwiegend die reproduktive Aktivität wider. Sie blieben gleich hoch bei Männchen, die von einem weniger aktiven zu einem aktiveren Phänotyp wechselten und sanken bei Männchen, die ihren Phänotyp in die andere Richtung wechselten. Jedoch sind die Ergebnisse nicht völlig übereinstimmend, denn überraschenderweise sanken auch die T Konzentrationen der Männchen, die über die gesamte Reproduktionsperiode territorial sind, obwohl sie anhaltend hohe reproduktive Aktivität und Aggressivität zeigten.

Die Ergebnisse von Kapitel 3 zeigen, daß das HPA-System von Meerechsen bereits auf geringe Umweltveränderungen reagierte. So bestand vermutlich ein Zusammenhang zwischen dem CORT Anstieg der Männchen im Laufe der Reproduktion und der zeitgleichen Klimaveränderung. Während der Reproduktionsperiode kommt es durch den Wechsel von Regen- zur Trockenzeit normalerweise zu ansteigenden Wassertemperaturen und damit zu geringerem Algenwachstum, der Hauptnahrungsquelle von Meerechsen. Eine Abnahme der körperlichen Konstitution aller Männchen im Laufe der Reproduktion unabhängig vom Phänotyp bestätigte diese Vermutung. Demnach schien CORT, durch die Nahrungsknappheit angeregt, Energiereserven zu mobilisieren. Solche Umweltveränderungen sind möglicherweise auch der Grund für die Aufgabe von Territorien und für einen Phänotypwechsel. Zwar zeigte T kein übereinstimmendes Muster im Zusammenhang mit reproduktiver Aktivität, meine Ergebnisse deuten aber darauf hin, daß T alle Männchen für die Reproduktion vorbereitet hatte, einschließlich der Männchen, die sich zu Beginn der Fortpflanzungszeit als nichtreproduktive Bachelor verhielten. Diese hatten anfangs ebenfalls höhere T Werte als zum Höhepunkt der Reproduktionsperiode. Ich schließe daraus, daß die Initialisierung reproduktiven Verhaltens von T gesteuert wird und daß T einen Wechsel zwischen den Phänotypen innerhalb einer Reproduktionsperiode unterstützt.

Neben natürlichen Veränderungen der Umwelt erleben Meerechsen seit Ende des 19. Jahrhunderts Veränderungen durch den Einfluß des Menschen, vor allem die neue Bedrohung durch eingeführte Prädatoren. Ob die Spannbreite der Reaktionsnorm von Meerechsen eine entsprechende Anpassung der Prädationsvermeidung an den erhöhten Prädationsdruck zuläßt, ist für Naturschutzprogramme von großer Bedeutung. In **Kapitel 4** verglich ich deshalb das Fluchtverhalten und die hormonelle Streßreaktion zwischen Meerechsenpopulationen an Orten mit und ohne eingeführte Prädatoren. Ziel war es zu klären, ob und wie schnell Meerechsen ihr Verhalten und/oder ihre physiologische Reaktion durch Anwesenheit von Prädatoren verändern. Ebenso testete ich das Fluchtverhalten und die hormonelle

Streßreaktion auf individuelle Unterschiede bezüglich Alter und Geschlecht. Ich bestimmte FIDs nach simulierter Prädatorannäherung (Annäherung des Experimentators) und CORT Konzentrationen (basale und streßinduzierte Werte). Ich erwartete, daß FIDs und CORT Konzentrationen bei Meerechsen an Orten mit eingeführten Prädatoren zunehmen. Individuelle FID und CORT-Unterschiede sollten außerdem dem jeweiligen Prädationsrisiko der Individuen entsprechen.

Tatsächlich reagierten Meerechsen unterschiedlich auf die simulierte Prädatorannäherung, je nachdem, ob sie eingeführten Prädatoren ausgesetzt waren oder nicht. FIDs waren höher an Orten mit Hunden und Katzen. Außerdem hatten sie erhöhte streßinduzierte CORT Konzentrationen. Der Faktor Alter hatte ebenfalls starken Einfluß sowohl auf die Varianz des Fluchtverhaltens als auch auf die Varianz der basalen und streßinduzierten CORT Werte innerhalb einer Population, d.h. Juvenile, welche aufgrund ihrer kleineren Körpergröße vermutlich stärker von Prädation bedroht sind als Adulte, zeigten höhere FIDs und CORT Konzentrationen.

Insgesamt zeigen die Ergebnisse von Kapitel 4, daß Meerechsen eine gewisse Plastizität in Fluchtverhalten und hormoneller Reaktion aufwiesen und dadurch in der Lage waren, Verhalten und Physiologie an neue Gefahren bis zu einem gewissen Grad anzupassen. Die Verhaltensanpassungen waren aber so gering, daß sie nicht ausreichend waren, um Prädation ausreichend zu reduzieren und um eine langfristige Aufrechterhaltung der Population zu gewährleisten.

Abschließend beschäftigte ich mich mit der Frage, ob das geringe Fluchtverhalten der Meerechsen mit einer fehlenden Aktivierung der HPA-Achse, die normalerweise bei Gefahr ausgelöst wird, direkt verbunden ist. In **Kapitel 5** untersuchte ich neben dem Fluchtverhalten (Messung von FIDs) die Sensitivität der HPA-Achse bei akuter Störung von Meerechsen an Orten mit unterschiedlichem Prädationsdruck und von Individuen, die zuvor negative Erfahrung durch ein Fangereignis gemacht hatten. Akute Störung wurde experimentell durch 15 Minuten Verfolgung (ohne körperlichen Kontakt) ausgewählter Meeresechsen durch den Experimentator verursacht. Nach 15 Minuten wurde das Tier gefangen und eine Blutprobe für die CORT Messungen entnommen.

Bei Meerechsen, die an Orten ohne oder mit mittlerem Prädationsdruck leben, stieg CORT nach experimenteller Störung nicht an. Dagegen zeigten Meerechsen auf San Cristobal, einem Ort mit starker Prädation durch Hunde, einen durch die Experimentator-Verfolgung ausgelösten deutlichen CORT Anstieg. Bei Meerechsen mit vorheriger negativer Erfahrung durch Fang konnte ebenfalls ein Ansteigen von CORT bei experimenteller Störung beobachtet werden, jedoch nur bei Individuen an Orten mit eingeführten Prädatoren.

Vermutlich beeinflußte die Anwesenheit von Prädatoren Meerechsen dahingehend, daß bereits durch einmalige negative Erfahrung eine Aktivierung der HPA-Achse bei folgender experimenteller Störung ausgelöst wurde. Aufgrund der Fähigkeit des HPA-Systems, auf verschiedene Stressoren zu reagieren, scheinen Meerechsen die Anpassungsfähigkeit der adrenalen Reaktion trotz des geringen Prädationsdrucks über Millionen Jahre bewahrt zu haben. Die hier vorliegenden Ergebnisse zeigen,

daß das HPA-System seine Aktivität schnell wieder zurück erlangen kann, wenn neue Bedrohungen auftauchen. Die Erhöhung der FIDs nach einmaliger negativer Erfahrung war jedoch sehr gering. Es ist daher von einer hohen phänotypischen Plastizität in der adrenalen Reaktion, aber einer geringen Plastizität im Fluchtverhalten bei Meerechsen auszugehen.

SCHLUSSFOLGERUNG UND FAZIT

Die auf den Galápagos Inseln zu beobachtende „Zahmheit" der Meerechsen steht vermutlich mit einer fehlenden Aktivierung des HPA-Systems in Verbindung, das normalerweise bei potentieller Gefahr aktiviert wird. Dies wiederum ist wahrscheinlich die Folge des sehr geringen Prädationsdrucks auf Galápagos in einem Zeitraum von 5-15 Millionen Jahren. Allerdings sind erste Anpassungen im Fluchtverhalten der Meerechsen durch die eingeführten Prädatoren zu beobachten. Diese Anpassungen sind jedoch noch zu gering, um dem neu aufkommenden Prädationsdruck effektiv Stand halten zu können. Dies zeigt sich vor allem in der hohen Mortalitätsrate durch eingeführte Prädatoren. Die adrenale Streßreaktion der Meerechsen zeigt hohe Plastizität, d.h. sie stieg bei Individuen an Orten mit eingeführten Prädatoren und nach einmaliger negativer Erfahrung deutlich an.

Die stark reduzierte oder fehlende Aufmerksamkeit und Scheu von Inselarten wird weltweit als Hauptgrund für deren abnehmende Populationsdichten auf Inseln mit eingeführten Prädatoren angesehen. Meine Ergebnisse zu den Limitationen der Spannbreite in Bezug auf Reaktionsnormen und der physiologischen Mechanismen von Fluchtverhalten bei Meerechsen liefern Informationen für die Planung von Schutzprogrammen gefährdeter Arten allgemein. Demnach erfolgt bei „zahmen" Inselarten eine Anpassung an neue Bedrohungen zwar relativ schnell, aber eben nur in geringem Maße. Diese Limitation der Spannbreite in Bezug auf Reaktionsnormen im Fluchtverhalten geht vermutlich mit reduzierten physiologischen Prozessen einher. So blieb die adrenale Reaktion während akuter Störung bei Meerechsen aus und wurde erst nach vorheriger negativer Erfahrung aktiviert. Diese Erkenntnis liefert nützliche Informationen für das Antrainieren von Fluchtverhalten bei in Gefangenschaft aufgewachsenen Wildtieren im Rahmen von Auswilderungsprogrammen. Konkret bedeutet das, daß ein Training bei solchen Arten zwar schnell ansprechen wird, vermutlich aber aufgrund fehlender adrenaler Reaktionen niemals die Effizienz erreichen wird, um Prädation zu reduzieren. In diesem Fall würden Auswilderungsprogramme nur Sinn machen, wenn vor der Auswilderung potentielle Prädatoren in der natürlichen Umgebung eliminiert werden.

Chapter 1

The influence of life history stages on flight initiation distance in naïve Galápagos marine iguanas

Silke Berger, Martin Wikelski,
Elisabeth K.V. Kalko, and Thomas Rödl

1.1 ABSTRACT

Flight, which is a common anti-predator response in most vertebrate species, can vary depending on the life-history stage of an individual associated with its predation risk. Thus, animals trade off between risk of capture and costs of escape according to the economic escape hypothesis that states when prey should initiate flight from approaching predators. We chose the Galápagos marine iguana to test the applicability of this hypothesis for island species that live under relaxed selection by predation. We measured flight initiation distance (FID) in marine iguanas and linked it to different age class, sex, and male reproductive phenotype. Since physiological constraints, such as body temperature, can have strong influence on escape behavior in ectotherms, we measured FIDs of individuals with different body temperatures to detect possible correlations. We also investigated the ability of marine iguanas to learn and adjust anti-predator response by negative experience. FID differed with age in relation to the relative vulnerability of the individuals to the predation threat from a native predator, the Galápagos hawk. We also found differences in FID according to sex and season. However, FIDs did not differ in males of the three reproductive phenotypes. Furthermore, in contrast to mainland species, marine iguanas lacked any adaptations in FID when slowed down by lower body temperatures. Surprisingly, marine iguanas increased FID after just one negative experience. The adjustments, however, were context-dependent and only occurred during the reproductive season. In conclusion, the economic escape hypothesis did not apply for marine iguanas. Overall, the observed adjustments to predation threat were very small and only sufficient for successful escape in island scenarios with low predation pressure. This can be seen as an adaptation of a species living in a predator-poor environment thus minimizing or avoiding costs that would have been otherwise caused by unnecessary escape.

1.2 INTRODUCTION

In nature, most animals live under heavy risk of predation and have evolved anti-predator strategies including vigilance and escape (Schall & Pianka 1980). However, anti-predator behavior may incur certain costs such as an animal that vacates its territory and subsequently looses opportunities for foraging and reproduction (Díaz-Uriarte 2001; Lima & Dill 1990; Walther 1969). Therefore, species that are under threat of predation have to balance risks and costs of escape against predation pressure as outlined in the optimal escape theory by Ydenberg and Dill (1986). This is illustrated for instance by hungry animals that need to balance anti-predator against foraging behavior (Lima et al. 1985).

In general, the decision when to flee differs with distinct life-history stages. For example, juveniles of some vertebrate species are warier than adults as a result of their smaller body size and concomitantly higher vulnerability to predators. Interestingly, this does not apply to all species (Cooper Jr. & Vitt 1985; Greene 1988; Whiting et al. 2003). Furthermore, anti-predator behavior can vary with sex (Clutton-Brock 1991; Fitch 1999), e.g. male lava lizards *(Tropidurus albemariensis)* have a shorter approach distance than females, possibly as a result of different body size (Snell et al. 1988). Reproductive behavior also may influence predator avoidance, e.g. territorial defense can be in conflict with predator avoidance, because a territorial resident that is hiding cannot simultaneously monitor its territory or defend it from conspecific intruders (Díaz-Uriarte 1999).

In ectothermic species, anti-predator responses are often temperature dependent because body temperature determines the animals` ability to escape from a predator (Shine et al. 2000). As an example, the lizard *Anolis lineatopus* increased approach distance when temperatures decreased, probably to reduce the risk of predation if slowed by low body temperatures during a predator attack (Rand 1964; in other Anolis species: Cooper 2006). Cooper (2000) found the opposite in keeled earless lizards *(Holbrookia propinqua)* where substrate temperature and approach distance were positively correlated. However, the lizards remained closer to their refuge after they had emerged in the morning, what gave them a better chance for escape during the cooler morning hours when body temperature is low.

On many islands, however, animals experience no predation pressure because of a lack of natural enemies (Beauchamp 2004). Thus, island species are often extraordinarily tame relative to their conspecifics on the mainland (Stone et al. 1994). Generally, studies have shown that relaxed selection by predation on animals living on islands, caused by evolutionary isolation from predators, profoundly influences their morphology, physiology and behavior (Blumstein et al. 2000). In particular, island species face fewer economic decisions and trade-offs between anti-predator behavior and other requirements such as territorial behavior or feeding, than their mainland relatives. We, therefore, hypothesize that anti-predator behavior of animals on largely enemy-free islands is not or only little influenced by factors such as age, sex, reproductive strategies or body temperature that are known as trade-offs regarding minimization of predation risk.

The marine iguana *(Amblyrhynchus cristatus)* that is endemic on the Galápagos islands is an ideal model to study anti-predator behavior of island species and the factors acting upon it. In contrast to their marine environment, where predation pressure probably occurs by sharks, marine iguanas experience relaxed selection by predation on land. Although, the Galápagos hawk *(Buteo galapagoensis)* is the most important native terrestrial predator of marine iguanas that mainly hunts nesting females (Laurie & Brown 1990), predation pressure by hawks is low. Furthermore, lava herons *(Butorides sundevalli)* sometimes catch hatchlings. Therefore, wariness of marine iguanas is very low and anti-predator responses are minor. Nevertheless, several factors such as temperature, age, sex or social status, can influence marine iguanas' anti-predator behavior. As an ectothermic animal and the only sea going lizard, marine iguanas show significant variations in body temperature during the day because they can cool down from 40°C to 25°C (Bartholomew 1966) during foraging in the sub- and intertidal zone and during the night. To compensate for this loss in body temperature, they perform behavioral thermoregulation (Bartholomew 1966). Age is positively correlated with body size in marine iguanas due to continuous growth, like in many other reptiles, and thereby possibly associated with their vulnerability to predation. Thus, younger individuals are more threatened by predation because of their smaller body size than adult, larger individuals. In addition, marine iguanas show a strong sexual dimorphism in body size, which, in turn, can cause sex related differences in anti-predator behavior. Furthermore, reproduction per se also can have a strong impact on anti-predator responses as male iguanas exhibit four reproductive phenotypes (bachelor, sneaker, satellite, and territorial), which differ in morphology, physiology and behavior (Carpenter 1966).

All these morphological and behavioral traits help to test whether animals on enemy-free islands follow the same rules and constraints on anti-predator behavior as mainland species. Island species that live in an environment with limited resources may reduce unnecessary escape responses possibly to decrease energetic costs of physical activity (McNab 1994). Energy conservation can contribute to an evolutionary loss of structure and behavior, such as the evolution of the flightless condition in birds (McNab 1994) or the low wariness in most island species.

Since marine iguanas face low predation threat, trade-offs between anti-predator response and other requirements associated with distinct life-history stages should be less crucial and thus less pronounced. Therefore, factors such as age, sex, and social status should not have a significant impact on anti-predator behavior. For the same reason, we also expected that anti-predator responses of ectotherm marine iguanas are not influenced by body temperature. Furthermore, the question arises whether island species adjust their anti-predator response to novel threats, e.g. introduced predators that occur on some of the Galápagos islands, and thus react more strongly for instance after negative experiences.

In summary, the overall aim of this study was to determine the influence of selected factors (age, sex, social status, temperature and experience) on the escape response of marine iguanas. We measured the response as the distance an investigator can approach before escape is initiated (FID =

flight initiation distance) (Martín & Lopez 1995). We then tested the possible association of FID with (1) body temperature fluctuations throughout the day, (2) reproductive strategies, (3) age effects, (4) sex differences during male reproductive season and female nesting season, and (5) previous experimentally induced negative experiences at the peak of male reproduction and during non-reproductive period.

1.3 METHODS

1.3.1 Study site

We investigated anti-predator behavior in a "naïve" island reptile species, the Galápagos marine iguana *(Amblyrhynchus cristatus),* in November/December 2003 and 2004 during the male reproductive period and in February 2005 during the female nesting period. The study was conducted on Santa Fé, an uninhabited island of the Galápagos archipelago with low terrestrial predation threat.

Our field site, 'Miedo' (0°50′S, 90°02′W), was located on the southeastern coastline of Santa Fé island, where marine iguanas live in large, dense aggregations. The coastline consists of extended inter-tidal zones with macrophytic marine algae exposed during low tide, on which marine iguanas feed exclusively (Trillmich & Trillmich 1986; Wikelski & Trillmich 1994). Lava rocks above the intertidal zone offer sites where marine iguanas often have extensive sun-baths after foraging in the inter- or subtidal zone.

1.3.2 Animals

As the only sea-going lizard, marine iguanas regulate their body temperature while basking on black lava rocks to absorb the heat (Bartholomew 1966). On land, marine iguanas strive for an optimum of 35.5°C body temperature. Body temperature, however, can drop to 10°C in the cold waters (20 – 25°C) of Galápagos (Bartholomew 1966; Bennett et al. 1975) as well as during the night when marine iguanas often sleep in large aggregations to conserve heat (Boersma 1982).

Although predation is generally very low, there is some predation pressure on marine iguanas as nesting females are threatened by hawk attacks. On Santa Fé, females move during the nesting period to sand and silt areas approximately 100 meter inland from the coast for 1 to 4 weeks in January/February (Carpenter 1966; Rauch 1988). Females are largely unprotected from potential predator attacks when digging nest holes, during egg-laying and while defending the nests. During this time, females constitute an easy prey for the Galápagos hawk (Wikelski pers. comment). Furthermore, females could be more threatened by hawks due to their smaller body size than males. Males and females can be distinguished by apparent morphological differences. Males have elongated spines and a wider neck and head than females. Moreover, males show red skin coloration on the back, particularly during reproduction, while females are dark gray (Carpenter 1966).

During reproduction, which starts in mid-October for males and lasts until mid-January, males may be in conflict between wariness and reproductive activity. Copulation rate peaks from mid to end of December (Carpenter 1966; Partecke et al. 2002). Male marine iguanas are polygynous and display in mating aggregations. Four male phenotypes are classified that differ in morphology and behavior. The phenotypes consist of territorials, satellites, sneakers and bachelors (Carpenter 1966; Eibl-Eibesfeldt 1966; Wikelski et al. 1996). Whereas territorial males defend mating territories, satellite males roam around and try to force copulations with females (Wikelski et al. 1996). Territorials are the biggest and most colorful males. Sneakers look and behave like females but try to sneak copulations with females. Bachelor males are not reproductively active. They do not show coloration and spend most of the season grouped with other bachelors outside clusters of reproducing males (Dellinger 1990; Laurie 1989; Wikelski et al. 1996).

1.3.3 Measuring anti-predator behavior by flight initiation distance (FID)

We applied a standard method developed for the study of anti-predator behavior in lizards (Bulova 1994; Burger & Gochfeld 1990; Cooper 1998; Cooper 1997; Cooper 2000; Martin & Lopez 1999) to measure FIDs of marine iguanas. In our protocol, we first selected immobile animals and then walked directly towards a selected individual at a slow pace (approx. 0.5 m/sec) without stopping. When an animal moved for at least its body length (snout vent length = measured from snout to cloaca) as a response to our approach, a marker (cloth) was dropped to document the flight initiation distance. We also distinguished between response types, i.e. whether an animal ran away or hid into crevices. We noted when crevices were available but the terrain was relatively homogeneous.

Prior to the experimental approach, we recorded sex, which is easy to distinguish in adult animals due to obvious morphological sex differences such as coloration, shape of head and spines, and body size. Furthermore, we used morphological and behavioral differences to determine the reproductive phenotype of the males, that is territorial, satellite or bachelor, and estimated snout-vent length (SVL) to assess the age class (hatchlings < 200 mm; juveniles = 200 – 300 mm; adults > 300 mm) of the selected individuals.

We always wore lightly colored clothing to minimize warning effects on anti-predator behavior. Furthermore, we did not wear sunglasses during controlled approaches because black iguanas *(Ctenosaura similis)* showed higher wariness when a large eye mask was worn during approaches (Burger et al. 1991).

1.3.4 Testing the influence of various factors on FID

First, we investigated the relationship between body temperature and FID of marine iguanas. Measurements were conducted between 6:20 and 18:30 hours. Only adult males during the non-reproductive season (mid February to beginning of March 2005) were chosen for this experiment to control for possible effects of sex, age and season. To measure body temperature, animals were caught

with a bamboo pole and a noose after the FID measurement was completed. Noosing is a common capture technique in lizards and does not harm the animals (Bloomberg & Shine 1996). Body temperature of marine iguanas was measured by a digital thermometer to the nearest 0.1 ºC by placing it in the cloacal area for ca. 10 seconds.

Second, we compared FID between marine iguanas of three male reproductive phenotypes (bachelor, satellite and territorial males) and three age classes (hatchlings, juveniles and adults), and between sexes during male reproductive period and female nesting period. We included only territorial males and adult females in the experiment to determine sex differences in FID during male reproductive period. During female nesting period, we did not differentiate between male reproductive phenotypes because males cannot be distinguished morphologically or behaviorally during this time.

Furthermore, we compared anti-predator behavior between "naïve" and "experienced" marine iguanas during and after male reproductive season. Experienced animals had been exposed to catching and handling three to four weeks prior to the anti-predator behavior measurements, whereas naïve animals had no contact to humans before. The comparison included only adult male marine iguanas.

We did not measure body temperature during all experiments to avoid unnecessary disturbance. Furthermore, we did not measure anti-predator behavior during the early morning and late afternoon, when body temperature in marine iguanas is low, in order to eliminate reduced responsiveness. During male reproductive season from end of October and beginning of January in 2003 and 2004, daytime air temperatures ranged between 22.8ºC and 28.6ºC (mean ± 1SE = 26.2 ± 0.1) and were somewhat lower than during the nesting season between end of January and end of February in 2005, when they ranged between 24.7ºC and 31.8ºC (mean ± 1SE = 27.1 ± 0.2).

All measurements and behavioral experiments on iguanas were done in accordance with the guidelines of the American Society of Ichthyologists and Herpetologists and were approved by the Princeton Institutional Animal Care and Use Committees according to NIH standards for the use of animals in research. They were applied on-site with the permission of the Galápagos National Park Service, Republic of Ecuador.

1.3.5 Statistics

Linear regression analysis was used to assess the relationship between FID and body temperature. We compared FID between male marine iguanas of different reproductive phenotypes and age classes with Kruskal-Wallis tests. Sex differences in FID and treatment effect on FID (experienced/naïve) were tested using independent t test and Mann-Whitney U test, respectively, for male reproduction period and female nesting period separately. Whether marine iguanas of different age classes show differences in the frequency of separate hiding strategies was tested with a Chi-square test and Phi Cramers V coefficient. Tests were two-tailed, with $\alpha = 0.05$. Data are reported as mean ± 1SE unless otherwise noted.

1.4 RESULTS

Our results showed a tendency of a positive linear relationship between FID and body temperature (BT) in marine iguanas (FID = $0.08 \times$ BT - 0.58, $F_{1,67}$ = 1.795, p = 0.077, r = 0.082; Fig. 1). Marine iguanas tended to show larger FIDs at higher body temperatures.

Age influenced anti-predator response as marine iguanas of different age classes showed differences in FID (H = 7.39, df = 2, p = 0.025, Fig. 2 A). In contrast, the FIDs of hatchlings and adults were lower than of juveniles (Dunn's Multiple Comparison test, juveniles vs adults p < 0.05). Furthermore, juveniles hid significantly more often into crevices than adults and hatchlings (hatchlings: 6.67 %, n = 15; juveniles: 12.5 %, n = 24; adults: 0 %, n = 205, Chi-square = 23.324, df = 2, p < 0.001, Phi Cramers V = 0.309, p < 0.001).
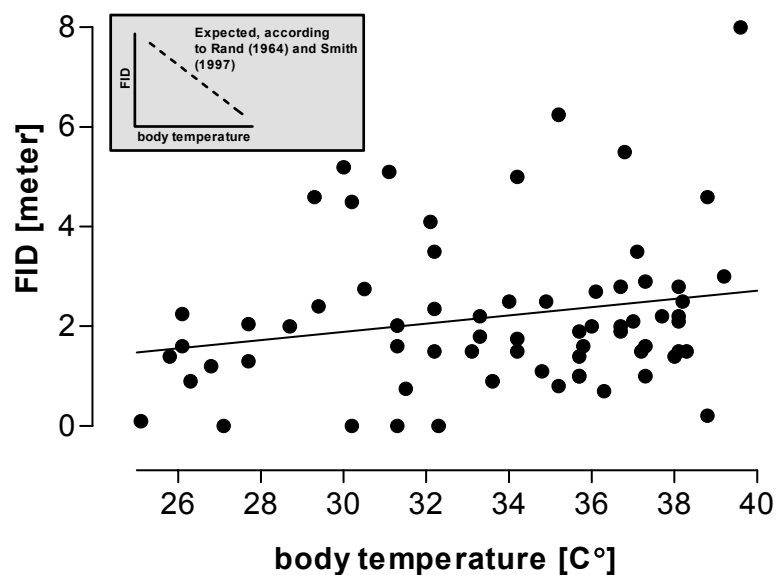
Fig. 1 Linear Regression between Flight Initiation Distance (FID) and body temperature in adult male marine iguanas after the reproductive season (solid line) (n = 69, p = 0.077) and expected correlation (dashed line) between FID and body temperature in reptiles according to findings from previous studies (Rand 1964, Smith 1997, graph in gray box).

FID did not differ between bachelor, satellite and territorial males during the male reproductive season (H = 1.07, df = 2, p = 0.585; Fig. 2 B). It differed between sexes only during the female nesting season, when the FIDs of female marine iguanas were significantly higher than those of males (t = -2.106, p = 0.038; Fig. 3 B). During the male reproductive season, both sexes had equal FIDs (t = -0.25, p = 0.805; Fig. 3 A).

In male marine iguanas, which had experienced catching and handling three to four weeks before, anti-predator measurements showed higher FIDs than in naïve males but only during the male reproductive season (t = -3.839, p = 0.001; Fig. 4 A). After the male reproductive season, the opposite effect occurred. Naïve male marine iguanas fled already at larger distances than experienced animals (Z = -2.125, p = 0.034; Fig. 4 B).
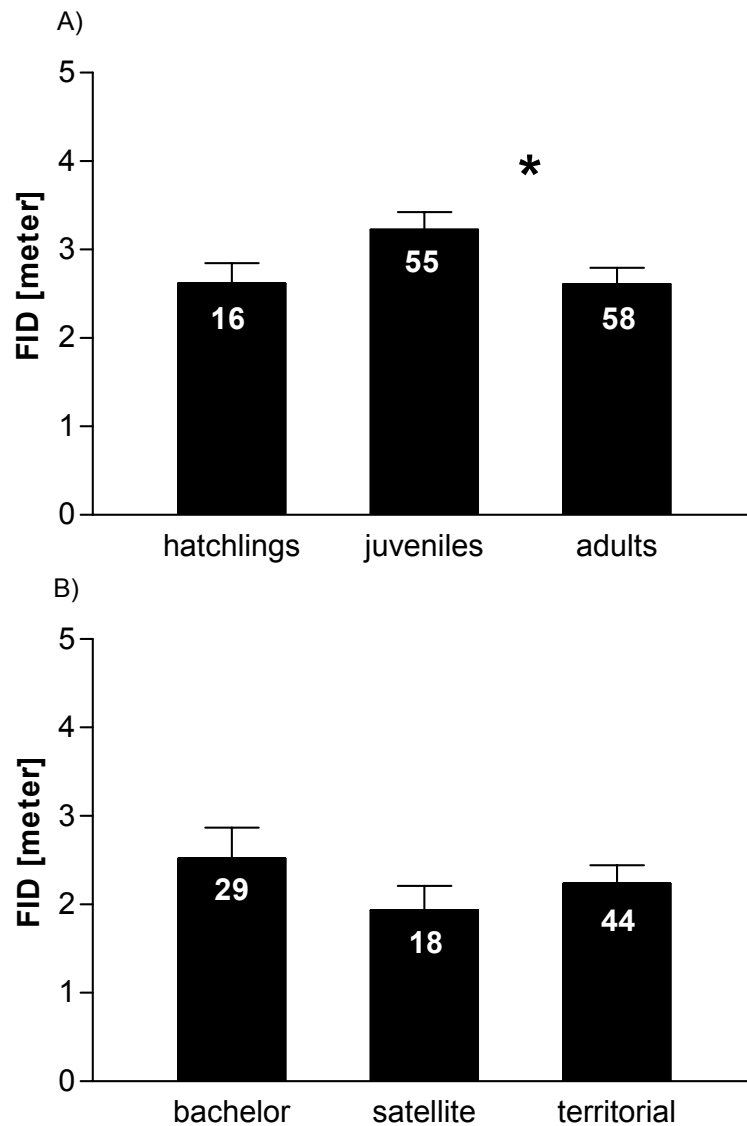

Fig. 2 Flight Initiation Distance (FID) of marine iguanas **A)** from three age classes (hatchlings = first year, juveniles = 2-3 years, adults >= 4 years) (Kruskal-Wallis test, p = 0.025, Dunn's Multiple Comparison test, age class 2 vs 3 p < 0.05) and **B)** of bachelor, satellite and territorial males during the peak of male reproductive season. Bars represent means ± 1SE.

A)
during peak of male
reproductive season

B)
after male reproductive season
(= nesting season)
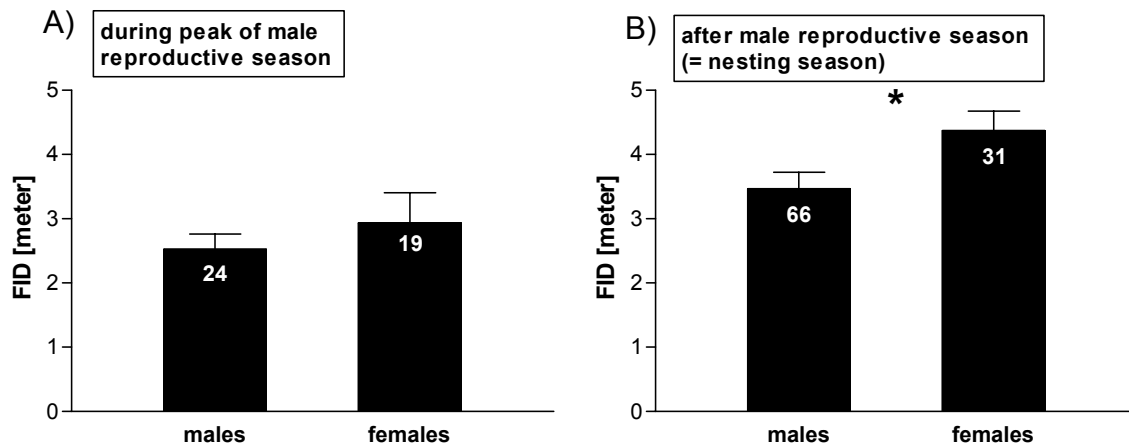
Fig. 3 Flight Initiation Distance (FID) of male and female marine iguanas A) during the peak of male reproductive season (independent t test, p = 0.222) and B) after male reproductive season (= female nesting season) (independent t test, p = 0.038). Bars represent means ± 1SE.

A)
during peak of male
reproductive season

B)
after male reproductive season
(= nesting season)
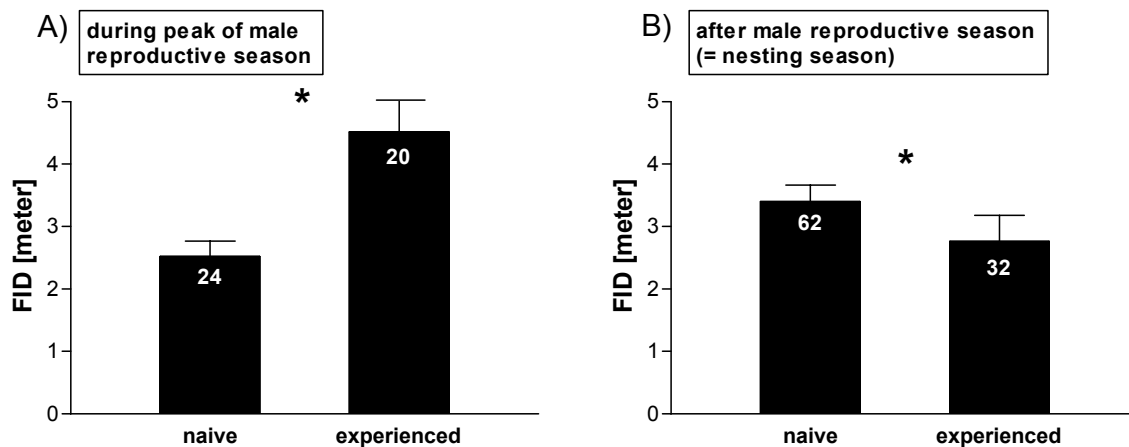
Fig. 4 FID of naive and experienced (three weeks after catching and handling) male marine iguanas A) during the peak of male reproduction (independent t test, p = 0.003) and B) after male reproduction (Mann-Whitney U test, p = 0.034). Bars represent means ± 1SE.

1.5 DISCUSSION

In the present study, we determined anti-predator behavior of an island species, the marine iguana, by measuring FIDs and its association with demographic factors, life-history stages and physiological constraints, in particular variation in body temperatures, which can impact the anti-predator responses by increasing their vulnerability to predation. We found only minor adjustments to lower the risk of predation, i.e. juveniles showed larger FIDs than adults, and females showed larger FIDs than males at times of elevated predation risk. However, the reproductive behavior of males did not influence escape response and body temperature was not a significant factor influencing FID.

1.5.1 Anti-predator behavior and body temperature

In contrast to our initial expectations, marine iguanas did not exhibit larger FIDs at lower body temperatures, although individuals with low body temperatures run significantly slower than individuals that are warm (Shallenberger 1970). Instead, marine iguanas tended to slightly increase FIDs at higher body temperatures. This is also in contrast to other studies where some lizards increased their approach distance to compensate for the slower movements, thus potentially reducing predation risk (Cooper 2006; Rand 1964; Smith 1997). Other lizards use alternative escape strategies, e.g. they hide in a secure retreat when they cannot maintain their body temperature within a preferred range (Rand 1964; Cooper 2000). In marine iguanas, however, we found that they hide only very rarely. Overall, we propose that the lack of an adequate strategy in marine iguanas to compensate for slowed-down movements at lower body temperatures is probably linked to the strongly reduced predation pressure on the islands.

1.5.2 Anti-predator behavior and reproductive behavior

Our results demonstrate that reproductive phenotypes do not influence anti-predator behavior in male marine iguanas. FID did not significantly differ between territorial, satellite and bachelor males. This is in our opinion another indicator for reduced predation pressure or the lack thereof, because a predatory attack usually creates a conflict for territorial males. If it runs away and hides to avoid predators, it cannot defend its territory from conspecific intruders (Díaz-Uriarte 2001). As an example, territorial water skinks *(Eulamprus heatwolei)* hid fewer times and returned faster to their basking position after a predator attack than floater males. Although this behavior facilitates territory defense and maximizes reproductive success, it incurs increased costs regarding predation (Stapley & Keogh 2005). At other instances, the decision to flee from an approaching predator may be deferred due to economic considerations according to optimal escape theory that determine a balance of risk and cost of escape (Ydenberg & Dill 1986).

While the decision to flee earlier at the expense of other requirements is beneficial in a predator-rich environment in terms of life-time fitness, in marine iguanas, however, benefits of escape are minor and thereby, such a trade-off is probably not of significance. Animals on islands can afford to minimize their anti-predator behavior and thus the costs associated with it in order to increase reproductive success without enhancing the risk of capture.

1.5.3 Anti-predator behavior and age

In predator-rich environments, predation is a strong selection pressure and is especially high during an animal's first year (Whiting et al. 2003). The smaller size of juveniles makes them much more vulnerable to predation than adults. In comparison to adults, young animals should alter their behavior in such situations to compensate for the greater risk of predation. However, previous studies failed to show a consistent pattern (Greene 1988; Martin & Lopez 1995; Whiting et al. 2003).

In our standardized experiments we found that juvenile marine iguanas had larger FIDs and hid more often than adults during simulated predator approaches. This is probably an adaptation to their higher vulnerability to predation by native Galápagos hawks, which prey mainly on young and female marine iguanas.

Surprisingly, hatchling marine iguanas showed similarly low FIDs than adults, although they are as vulnerable as juveniles or even more due to their smaller body size. Hatchlings might lack the experience and learning phase to respond appropriately to predation threat (Marcellini & Jenssen 1991), or they only flee when the predator is so close that the probability of being detected is higher than the risk of drawing attention because of running (Ydenberg & Dill 1986).

1.5.4 Anti-predator behavior and sex

We showed that female marine iguanas exhibited larger FIDs than males at times of nesting. In contrast, marine iguanas did not show significant sex differences during the reproductive period of males. However, whether the sex differences in marine iguanas are a consequence of differences in body size or a cause of distinct selective forces on males and females is unknown and was debated also for other vertebrate species (Shine et al. 2000; Snell et al. 1988). As an example, male Galápagos lava lizards have shorter FIDs than females, probably because costs of escape in terms of loosing a territory and, thereby, mating opportunities for reproduction are higher for males than for females (Snell et al. 1988).

The larger FIDs of female marine iguanas during the nesting period are probably an adjustment to the higher predation pressure on females by hawks during this time. Nesting and egg-laying female marine iguanas at the nesting sites are an easy prey for Galápagos hawks because females are not able to hold on with their claws on sandy surfaces of nesting sites as they do on rocks. Thus, a hawk can lift females from the surface. Furthermore, the Galápagos hawk on Santa Fé raise young during marine iguana's nesting time and feed them mostly with marine iguanas (DeVries 1976). However, as we measured the FIDs at the rocky shore, where females are less threatened by hawk predation than at the females' nesting sites, because we wanted to avoid disturbance of the nest holes, one might argue that site influenced the anti-predator behavior of females.

1.5.5 Anti-predator behavior and experience

Our results did not show an overall consistent association between anti-predator behavior and experience of the animals. During the reproductive period of male marine iguanas, males with negative experiences due to prior capture and handling exhibited significantly higher FIDs than naïve males. In contrast, during the non-reproductive season the reverse was true. Probably, the social context during reproduction causes the increase in FIDs in experienced males. In general, after an unsuccessful fight, inferior males may avoid future aggressive encounters, probably to save energy. Perhaps, a capture event by humans is perceived from a male as an unsuccessful fight. Therefore, an experienced male

may show the same response as an unsuccessful male after fighting, namely fleeing at a larger distance to avoid costs of being captured again.

Furthermore, elevated FIDs in marine iguanas during reproduction can be a result of higher adrenal and behavioral responsiveness caused by hormones such as testosterone and/or corticosterone, which are elevated during the reproductive period (Berger et al. 2005; Wikelski et al. 2004). Corticosterone can increase the reactivity to external stimuli (Badyaev 2005) and thereby lead to higher wariness in marine iguanas. In general, particularly corticosterone is capable of inducing irruptive behaviors such as activities that promote escape (Astheimer et al. 1995; Breuner et al. 1998). Thus, elevated adrenocortical responsiveness is considered as an adaptation to increase survival by removing the animal away from a stressor (Wingfield et al. 1998).

Overall, our results demonstrate that marine iguanas on a predator-poor island adjust FIDs according to their life-history stage, previous experiences and vulnerability to local predation threat. Those adjustments, however, are very minor and context-dependent and may only be adaptive in an island scenario such as the Galápagos. In a continental scenario, the adjustments in FID are probably not enough to avoid capture as shown by a recapture rate of 100 % during another experiment with experienced marine iguanas (Berger & Rödl unpublished data). We thus conclude that island lizards are highly vulnerable to introduced exotic predators because they are not able to respond appropriately to the new predation threat.

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Fig. 1 Measurement of flight initiation distance (FID), a standard method developed for the study of anti-predator behavior. First, an immobile animal is selected and then an investigator walks directly toward the individual at slow pace (approx. 0.5. m/sec) without stopping. When the animal moves at least over a distance equivalent to its body length (snout vent length) as a response of the investigators approach, a marker (small rag) will be dropped to document the FID.
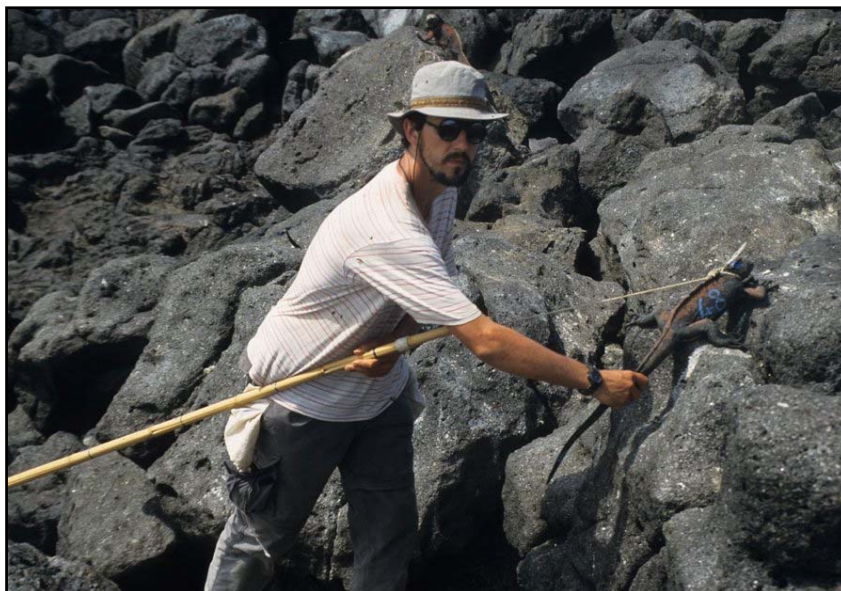
Fig. 2 Animals were caught with a noose attached to a bamboo pole after the FID measurement was completed. Noosing is a common capture technique in lizards and does not harm the animals.

Chapter 2

Corticosterone suppresses immune activity in territorial Galápagos marine iguanas during reproduction

Silke Berger, Lynn B. Martin II, Martin Wikelski, L. Michael Romero,

Elisabeth K.V. Kalko, Maren N. Vitousek, and Thomas Rödl

2.1 ABSTRACT

Individuals that display elaborate sexually selected characters often show reduced immune function. According to the immunocompetence handicap hypothesis, testosterone (T) is responsible for this result as it drives the development and maintenance of sexual characters and causes immunosuppression. But glucocorticoids also have strong influences on immune function and may also be elevated in reproductively active males. Here we compared immune activity using the phytohemagglutinin (PHA) skin test in three discrete groups of male marine iguanas *(Amblyrhynchus cristatus)*: territorials, satellites, and bachelors. Males of these three reproductive phenotypes had indistinguishable T concentrations during the height of the breeding season, but their corticosterone (CORT) concentrations, body condition and hematocrit were significantly different. Territorial males, the animals with the most elaborate sexual ornaments and behaviors, had lower immune responses and body condition but higher CORT concentrations and hematocrit than satellites or bachelors. To test directly CORT's immunosuppressive role, we elevated CORT by either restraining animals or additionally injecting CORT and compared their PHA swelling response with the response of free-roaming animals. Such experimental elevation of CORT significantly decreased immune activity in both restrained and CORT injected animals. Our data show that CORT can induce immunosuppression, but they do not support the immunocompetence handicap hypothesis in its narrow sense because T concentrations were not related to immunosuppression.

2.2 INTRODUCTION

Immune function is often compromised in males with the most elaborate secondary sexual traits (Verhulst et al. 1999; Zuk & Johnsen 2000; Zuk et al. 1995), but the proximate causes for this relationship are not well understood. It is often suggested that the androgen testosterone (T) is responsible for both exaggerated sexual traits and immunosuppression, as formalized by the immunocompetence handicap hypothesis (Folstad & Karter 1992). Trade-offs between T-related secondary sexual characters and immune activity exist because both are costly (Demas et al. 1997; Gustafsson et al. 1995; Hanssen et al. 2004; Lochmiller & Deerenberg 2000; Martin et al. 2003; Råberg et al. 2000; Sheldon & Verhulst 1996). Only high quality males can display elaborate traits and simultaneously fend off ubiquitous attacks by parasites and pathogens (Hamilton & Zuk 1982).

In support of the T-immunity trade-off, experimental studies in various vertebrates have found elevated T concentrations at the time when immune activity is reduced (Hillgarth & Wingfield 1997; Nelson & Klein 2000; Verhulst et al. 1999; Zuk et al. 1995). Experiments with T-implanted males have shown that T can directly cause immunosuppression as measured by a reduction in serum antibody titers to antigenic challenge (Duffy et al. 2002; Saad et al. 1990) and by a suppressed cell-mediated immune response (Duffy et al. 2002). In several experiments, ectoparasite loads were associated with immunocompetence (Olsson et al. 2000; Poiani et al. 2000; Salvador et al. 1996). T-implanted animals harboured relatively higher ectoparasite loads – a fact that was explained as a result of compromised immune function. Other studies, however, have been unable to confirm immunosuppression by elevated concentrations of plasma T. Saino et al. (1995) have shown that T-implantation resulted in higher levels of parasite infection, but could not find an obligatory immunosuppressive effect of T as measured by leucocyte counts and immunoglobulin concentrations. Further studies also found that neither antibody responses (Hasselquist et al. 1999; Lindström et al. 2001; Sapolsky et al. 2000) nor cell-mediated immune response (Greenman et al. 2005) were suppressed by above-normal concentrations of plasma T. The contradictory results of these T-implantation experiments suggest that the immune system is a very complex system and there may be no consistency in the effects of T on specific immune responses. Furthermore, the influence of T on immune function may be condition-dependent (Peters 2000). Peters (2000) found T-induced immunosuppression in superb fairy-wrens only under laboratory conditions; under natural conditions, individuals with the highest T concentrations had the strongest immune response. She suggested that T-induced immunosuppression is highly condition-dependent and only immunosuppressive when resource availability is standardized, such as in the lab. In the field, where differences in resource acquisition ability and hence in body condition exist, the relationship between T and immunocompetence is not consistent (Peters 2000).

While these results are exciting, other hormones may be important, and elevations in glucocorticoids may explain the apparent condition-dependent effects of T.

For instance, Casto et al. (2001) showed that male dark-eyed juncos *(Junco hyemalis)* implanted with T had increased concentrations of corticosterone (CORT), a steroid hormone whose circulating blood concentrations are increased during times of stress. Thus, whether the observed immunosuppression in his and other birds is actually due to T's direct effects on immunity or to T's influence on CORT production remains unclear. Indeed, Evans et al. (2000) also found elevated CORT concentrations after experimental T augmentation in house sparrows, which in turn were related to the degree of immunosuppression. After statistically controlling for the effect of CORT, T apparently enhanced the birds' ability to produce antibodies. A causal role for glucocorticoids in immunosuppression is conceivable as many studies have documented that circulating concentrations of corticosteroids are inversely correlated with immune function (Munoz et al. 2000; Nelson & Demas 1996; Saad & el Ridi 1988; Sapolsky 1992). Similarly, the administration of exogenous CORT provided strong evidence for CORT-induced immunosuppression in lizards *(Chalcides ocellatus)* and alligators *(Alligator mississippiensis)* (Morici et al. 1997; Saad et al. 1986). However, it is still unclear whether wild animals show immunosuppression when exposed to naturally occurring elevations of CORT in free-living animals.

In the present study, we used wild male marine iguanas *(Amblyrhynchus cristatus)* as a model system to investigate the interaction between reproductive behavior, body condition, immune function and steroid hormones. Because male marine iguanas differ in reproductive activity, which is probably associated with hormone concentrations, they are most suitable for these kinds of studies. Furthermore, endocrinological methods are already validated and well established in this species (Romero & Wikelski 2001), and ease of capture and their calmness minimizes handling times. The marine iguana is a polygynous reptile species where males display in mating aggregations. Three male types can be classified that differ in morphology and behavior: territorials, satellites, and bachelors (Carpenter 1966; Eibl-Eibesfeldt 1966; Moore et al. 2000; Wikelski 1996; Wikelski et al. 1996). Territorial males are large and the most ornamented group. During the reproductive season, they have distinct skin coloration and elongated dorsal spines. They frequently show ritualized head-bobbing, a typical reproductive behavior to attract females and threaten off rivals (Carpenter 1966; Wikelski et al. 2001). Territorial males, which defend clustered mating territories, are often surrounded by numerous females, and receive 95% of all copulations (Parsons 1993). Satellite males are smaller and less ornamented than territorial males. They try to force-copulate with females leaving male territories, a strategy that results in low reproductive success (Wikelski et al. 1996). In general, male marine iguanas do not participate in reproduction each consecutive year probably to replenish their energy reserves after reproductive efforts. Males that were territorial in previous reproductive seasons, but are now reproductively quiescent during the current year, are called bachelor males. They do not exhibit reproductive behavior or ornamentation, which is probably associated with low T concentrations and regressed gonads. They spend most of their time grouped with other bachelors outside clusters of reproducing males (Dellinger 1990; Laurie 1989; Wikelski et al. 1996).

At the beginning of the reproductive season when territories are being established, territorial males have the highest T concentrations, followed by satellites and bachelors. However, at the peak of reproduction, T concentrations decrease in territorial and satellite males and do not differ between reproductive phenotypes (Nelson 2003; Wikelski et al. 2004). We took advantage of this seasonal decrease in T concentrations and compared T-lymphocyte-mediated immunity in territorial, satellite, and bachelor males when they had similar T concentrations. The aim of our study was to test whether reproductively active males show immunosuppression in the absence of T differences and if other factors such as CORT play a more important role in compromising the immune system in these males. In addition, we measured the influence of experimentally elevated CORT concentrations on immune function in individual bachelor males. This allowed us to directly test whether CORT alone can suppress the immune system of marine iguanas.

2.3 METHODS

2.3.1 Field site and animals

Our field site, 'Miedo' (0°50′S, 90°02′W), is located on the southeastern coastline of Santa Fé island. Marine iguanas at Miedo live in large, dense aggregations. The coastline consists of lava rocks and extended inter-tidal zones with macrophytic marine algae exposed during low tide, on which marine iguana feed exclusively (Trillmich & Trillmich 1984; Wikelski et al. 1993; Wikelski & Hau 1995; Wikelski & Trillmich 1994). Lava rocks above the inter-tidal zone offer territories for males. This study was performed from December 11, 2002 to January 3, 2003 during the peak of the reproductive season. Individuals were caught with a noose and marked with synthetic paint to ease observation and recapture. Paint markings did not change iguana behavior or interactions in previous studies (Audet and Wikelski, unpubl. data).

2.3.2 Phenotypic comparisons

2.3.2.1 Male status

Males were classified as territorial, satellite, or bachelor by repeatedly observing their behavior for 10 minutes at different times of different days. A male was categorized as territorial if it exhibited frequent head-bobbing against other males and was found at least three times at the same location, successfully defending that location against rivals (Partecke et al. 2002; Wikelski et al. 1996). Satellite males generally head-bob less and are not consistently found at the same site. Males were classified as bachelors if they did not head-bob or engage in other obvious reproductive behaviors. We marked (a number with synthetic paint) and investigated a total of 49 individual males (24 territorials, 16 satellites, 9 bachelors).

2.3.2.2 Immune function

We measured immune activity using the phytohemagglutinin swelling response (PHA-test). PHA causes local swelling and edema, driven by mitogenesis and infiltration of immune cells into injected tissue (Goto et al. 1978). The PHA-test has been used increasingly in ecological studies of free-ranging birds and is supposed to be harmless to animals. Because to the best of our knowledge the PHA-test had never been used in iguanids and only once in lizards (Svensson et al. 2001), we tested our planned methodology in a pilot study in the lab conducted on green iguanas *(Iguana iguana)*, caught in the wild in Florida. We determined the lowest doses of PHA which induces a measurable swelling and confirmed that no obvious behavioral effects or indications of sickness were occurring. 5 mg PHA/ml induced a significant swelling peak 18 hours after injection. To ensure there was no species bias in our calibration, we re-validated our technique in 23 male marine iguanas using exclusively non-territorial males. In these animals, we injected 0.1 ml of 5 mg/ml PHA solution (PHA-P, Sigma L-8754) into the toe-web between the fourth and fifth toe of the right back foot. As a control, we injected 0.1 ml of pure saline solution into the toe-web of the left back foot. To determine peak swelling, we measured the thickness of the toe-web immediately before injection and 6, 12, 18, 24, and 48 hours after injection at the injection site to the nearest 0.05 mm using a pressure sensitive spessimeter (Te-clock, model SI-510).

After each measurement, animals were released and recaptured just prior to the next measurement period to allow us to obtain repeated PHA responses of free-living animals under natural conditions. We found that peak swelling in marine iguanas occurred 12 hours after PHA injection (Fig. 1). Handling during all measurements was kept at a minimum to prevent prolonged CORT increase, which may have reduced the immune response. For the ensuing experiments we applied the validated PHA-test in three male reproductive phenotypes by measuring the PHA swelling response 18 hours after injection to avoid swelling measurements during the night.

2.3.2.3 Hormone concentrations

We used the standard capture and restraint stress protocol (Romero & Wikelski 2001; Wingfield 1994) to quantify plasma CORT concentrations. Males were caught with a noose and an initial blood sample (within 3 min of capture) of about 1 ml was collected from the tail vein into sodium heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Sampling was only done from noon to late afternoon over 4 consecutive days to control for variation due to circadian and tidal cycles in circulating T and CORT (Nelson 2003; Woodley et al. 2000).

Body temperature was measured in the cloaca with a digital thermometer (accuracy 0.1°C) immediately after initial blood collection to control for its potential influence on CORT, and we excluded animals with body temperatures lower than 30°C. Iguanas were subsequently held in an opaque cloth bag for 30 minutes when a second blood sample was collected. Blood samples were stored cool for up to 6 hours in a thermos bottle on cooling gel packets and then centrifuged at 2000 g for 10 minutes.

Plasma was removed from each sample and stored at 4°C for 7 days in a gas-fueled cooling box until it could be frozen at -20°C. A subset of the plasma of initial blood samples was separated for T analysis and its volume was measured with a Hamilton syringe. This aliquot of plasma was stored in a tight vial containing 750 μl of 99.5% ethanol.

2.3.2.4 Body condition

We determined hematocrit by centrifuging two blood-filled capillary tubes from initial blood samples in a Mini Centrifuge (Compur, Bayer Diagnostika, Germany) at 1400 g for 3 minutes. We used the mean of the two samples as our estimate. Also, each iguana was weighed (to the nearest 10 g) and the snout-vent length (SVL) was determined to calculate a body condition index (($body\ mass/SVL^3)*10^6$) as a measure of the physical condition (Laurie, 1989). Iguanas die at indices of less than ≈ 25 and are maximally fat at indices of 60 (Romero & Wikelski 2001).

2.3.3 Stress-induced immunosuppression - an experimental approach

To test whether elevated CORT concentrations directly induce immunosuppression, we compared the PHA swelling response 6, 12, and 18 hours after immune challenge between three groups of bachelor marine iguanas: i) animals caught and held for 12 hours under restraint stress in an opaque bag (n = 9), ii) CORT-injected animals under the same restraint stress (n = 8), and iii) free-living animals, which were caught and released immediately after the treatment (n = 23). For groups i) and ii), we caught males in the late afternoon to ensure that animals did not miss a foraging period. Furthermore, we ran the experiment during the night to avoid holding animals for 12 hours in high ambient air temperatures.

After catching each animal, we used the standard stress protocol (see above) to obtain plasma baseline CORT concentrations and CORT responses. From this pool of animals, we randomly chose 9 animals for CORT injections (treatment) and 8 animals for pure peanut oil injections (control). After the 60-minute blood sample of the stress protocol, we injected each treatment animal with CORT diluted in peanut oil (200 μl solution / kg body weight for a dose of 1 mg CORT / kg body weight). After injection, we took additional blood samples for CORT analysis at intervals of 120, 240, 360, and 720 minutes after initial capture from both CORT and control injected animals; the PHA swelling response was also measured at 360, 720, and 1080 minutes after PHA injection without knowledge of the treatment group. All procedures on iguanas were conducted in accordance with the guidelines set forth by the American Society of Ichthyologists and Herpetologists and were approved by the Princeton and Tufts University Institutional Animal Care and Use Committees (accordant to NIH standards for the use of animals in research) and accomplished with permission of the Parque Nacional Galápagos.

2.3.4 Steroid assays

CORT and T were extracted with dichloromethane and analyzed by indirect radioimmunoassay (described in detail in Romero & Wikelski 2001; Wingfield & Farner 1975; Wingfield et al. 1992). We used T antibody, T 3003, from Wien Laboratories, Succasunna, NJ, and CORT antibody, B3-163, from Esoterix Endocrinology, Austin, TX. Each sample was assayed in duplicate with a small amount of radio-labeled steroid added to determine recovery. Intra-assay variation of the hormone assays was on average 3.8 % for CORT and 12 % for T. The detection limit was 0.13 ng/ml for CORT and 0.17 ng/ml for T. The alcohol-preserved samples were prepared for dichloromethane extraction as described in Tarlow et al. (2003).

2.3.5 Statistical analysis

We used univariate AN(C)OVA (Analysis of (Co)Variance) models and Tukey HSD post hoc pairwise comparisons to compare PHA swelling response, T, baseline CORT, CORT response, body condition, and hematocrit among male phenotypes. Linear regression models were used to investigate possible correlations among measured variables. Baseline CORT values were $\log_{10}$ transformed and hematocrit percentages were arcsine square-root transformed to achieve normality and homogeneity of variances of our data.

For analysis of data from the stress-induced immunosuppression experiment, we used unpaired students t tests to compare CORT concentrations between groups and paired t tests to compare CORT concentrations within groups between different time points. Differences in PHA swelling response between free-living animals, restrained, and CORT injected animals were tested with univariate ANOVA and Tukey HSD post hoc pairwise comparisons. 18 hours PHA swelling responses were $\log_{10}$ transformed to achieve homogeneity of variances. To test the PHA method, we compared the PHA swelling response with the PBS response (control) with paired t tests to determine if controls had significantly different (lower) responses than the PHA injected animals. Sample sizes for hormone analysis, body condition variables and PHA swelling responses differed among groups because not all of the males could be recaptured, bled and measured at all times. Data were analyzed with SPSS for Windows 10.0 and α was set to p = 0.05.

2.4 RESULTS

2.4.1 Validating the PHA swelling response

The PHA swelling response was strongest at 12 hours, compared to 6, 18, 24, and 48 hours (n = 23). At each time point, the PHA swelling response was significantly larger than the PBS swelling response (6 hours: t = 5.22, p < 0.001; 12 hours: t = 8.81, p < 0.001; 18 hours: t = 6.11, p < 0.001; 24 hours: t = 5.04, p < 0.001; 48 hours: t = 3.26, p = 0.008; Fig. 1).
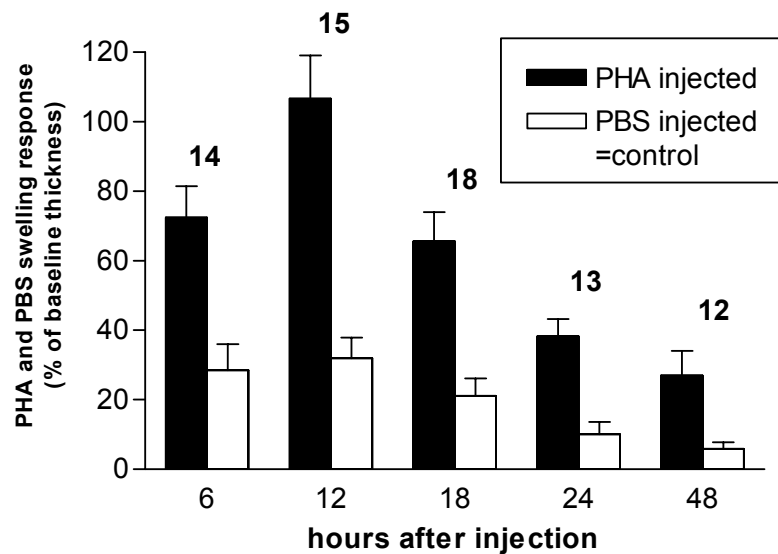
Fig. 1 PHA and PBS (control) swelling response in male marine iguanas 6, 12, 18, 24, and 48 hours after injection. Bars represent means ± 1SE. Numbers above bars indicate sample sizes.

2.4.2 Comparison between reproductive phenotypes

The PHA swelling response was significantly different between male types ($F_{2,37}$ = 4.4, p = 0.02). Territorial males exhibited a lower swelling response than satellites (Fig. 2 A). However, T concentrations were not significantly different between types (Fig. 2 B). Body temperature (ranged from 31.3 – 39.2°C) had a significant influence on baseline CORT ($F_{1,43}$ = 11.15, p = 0.002) and on CORT response ($F_{1,42}$ = 8.26, p = 0.006). We ignored any possible influence of body temperature on swelling response in our study because we predicted similar mean body temperatures during the PHA induced immune response over 18 hours. We found a difference between male types in baseline CORT ($F_{2,43}$ = 14.72, p < 0.001), but not in CORT response. Territorial males exhibited higher baseline CORT concentrations than satellites and bachelors (Fig. 2 C). Furthermore, the male types differed significantly in hematocrit ($F_{2,44}$ = 3.8, p = 0.03) and body condition ($F_{2,46}$ = 9.4, p < 0.001). Body condition was lower in territorials compared to satellites or bachelor males (Fig. 2 E). Territorial males had a significantly higher hematocrit than bachelors (Fig. 2 F). We found no significant differences between satellites and bachelors in baseline CORT, body condition index, hematocrit, or swelling response (Fig. 2). In sum, territorial males exhibited the lowest immune response, the highest baseline CORT, the lowest body condition index, and the highest hematocrit in comparison with the two other reproductive phenotypes.

Baseline CORT was negatively correlated with body condition index (F = 9.019, p = 0.004; Fig. 3 B). Furthermore, we found a negative linear regression between PHA swelling response and baseline

CORT (F = 6.12, p = 0.018; Fig. 3 A) and between PHA swelling response and hematocrit (F = 13.25, P = 0.001; Fig. 3 C).
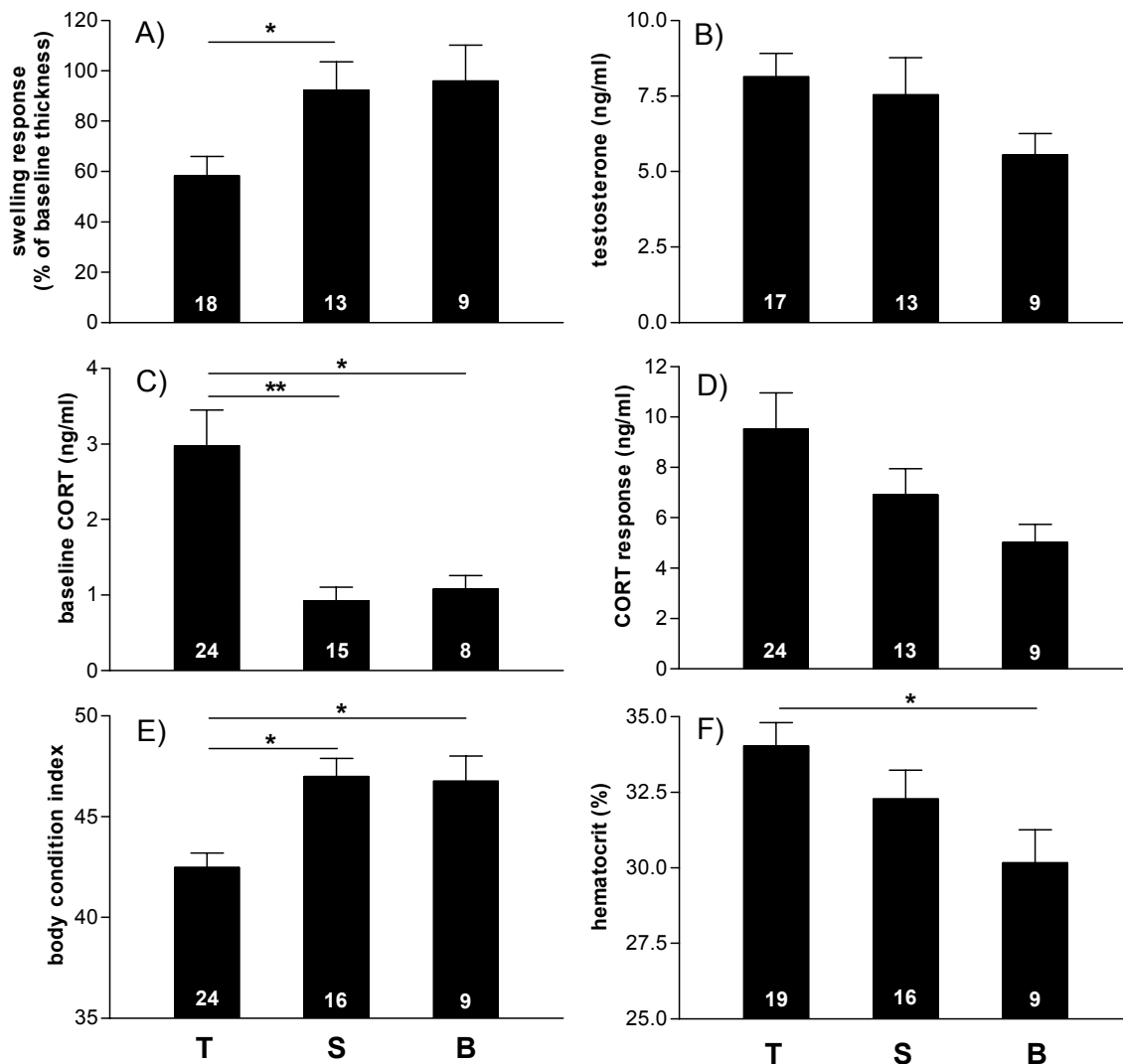
Fig. 2 A-F A) Swelling response, **B)** T, **C)** baseline CORT, **D)** induced CORT response, **E)** body condition index ((body mass/snout-vent length^3)*10^6), and **F)** hematocrit in male marine iguanas with different reproductive phenotypes: territorials (T), satellites (S), and bachelors (B). Bars represent means ± 1SE. Numbers in bars indicate sample sizes. Horizontal lines above bars indicate statistical significances for Tukey post hoc pair-wise comparisons: ** p < 0.01; * p < 0.05; other comparisons are not significant.

2.4.3 Stress-induced immunosuppression - an experimental approach

After 30 and 60 minutes restraint stress, CORT concentrations were significantly higher as compared to baseline concentrations (t = -6.87, p < 0.001; Fig. 4). Animals kept under 120 minutes restraint stress and injected with peanut oil (control) exhibited lower CORT concentrations than captive animals injected with CORT (t = -7.57, p < 0.001; Fig. 4). There was no significant change between the

60 and 120 minute samples in the control group, but CORT increased significantly after CORT injection (t = -7.26, p < 0.001; Fig. 4). During the 240 and 360 minutes of restraint stress, the CORT injected and oil injected group differed significant in CORT concentrations (240 minutes: t = -8.74, p < 0.001, 360 minutes: t = -7.36, p < 0.001; Fig. 4), but there was no longer a significant difference after 720 minutes. Both groups of males under restraint stress (with CORT or with oil injection alone) had a lower PHA swelling response 12 and 18 hours after PHA injection compared to free-roaming males (12 hours: $F_{2,25}$ = 5.10, p = 0.01; 18 hours: $F_{2,28}$ = 10.20, p < 0.001; Fig. 5). There was no significant difference in the swelling response between male types 6 hours after PHA injection. Thus, male marine iguanas with elevated CORT concentrations, either caused by restraint stress or CORT injection, exhibited a reduced immune responsiveness as compared to free-roaming animals.
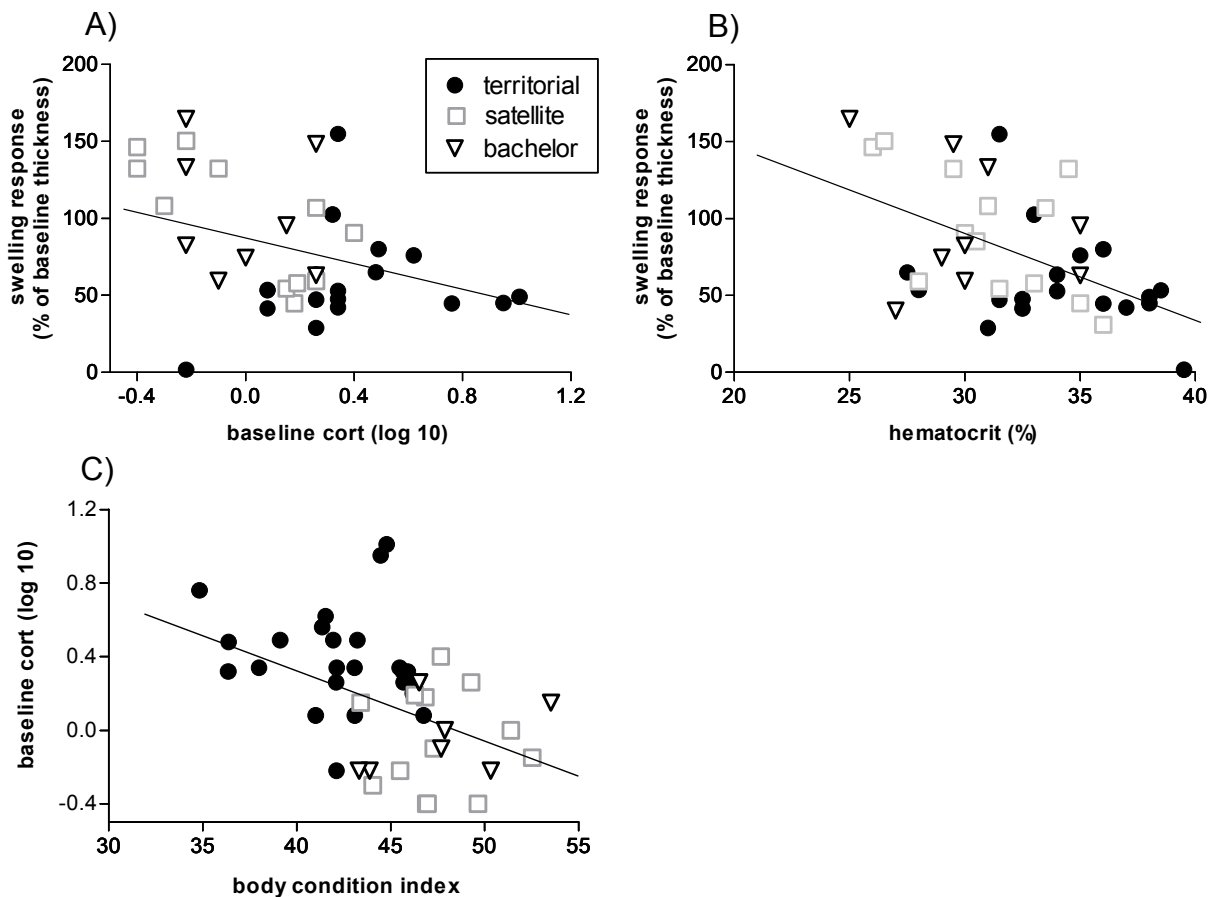
Fig. 3 A-C A) Swelling response correlates with baseline CORT concentrations (p = 0.018, n = 38), **B)** baseline CORT concentrations correlates with body condition (p = 0.004, n = 47), and **C)** swelling response correlates with hematocrit (p = 0.001, n = 40) in territorials, satellites, and bachelors. The linear regression models include males of all three reproductive phenotypes.
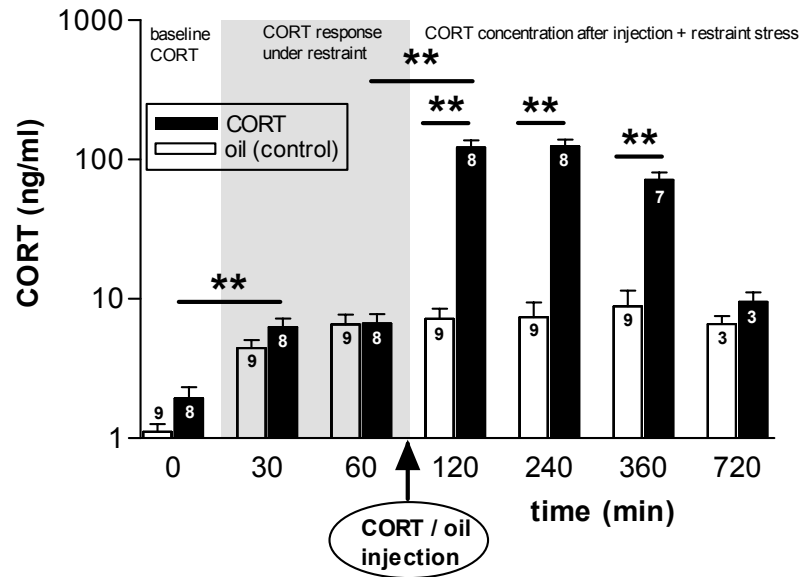
Fig. 4 Plasma CORT concentrations in male marine iguanas (bachelor males) before restraint stress (baseline CORT), under restraint stress (oil injected = control), and under restraint stress + CORT injection. Bars represent means ± 1SE. Numbers in bars indicate sample sizes. The symbols between bars indicate statistical significances: ** $p < 0.01$; * $p < 0.05$; other comparisons are not significant. For analysis paired t test and unpaired t test were used.
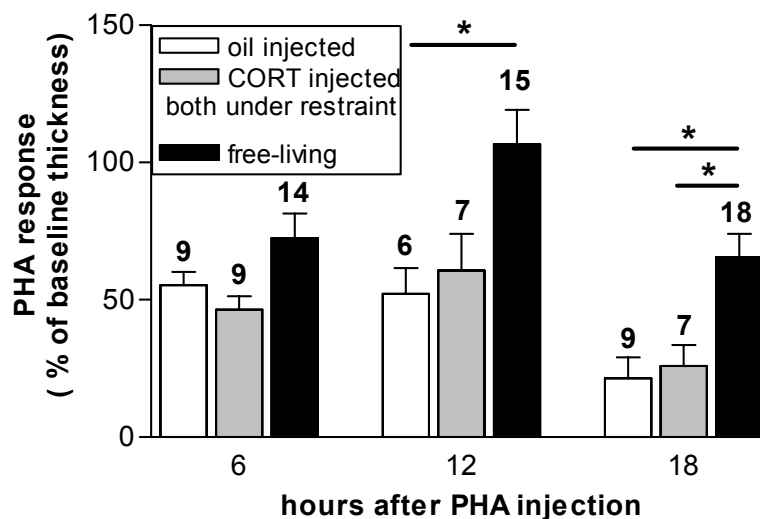
Fig. 5 PHA swelling response 6, 12, and 18 hours after PHA injection in male marine iguanas (bachelor) after oil or CORT injection (both under restraint) and in free-living male marine iguanas (bachelor). Error bars represent means ± 1SE. Numbers above bars indicate sample sizes. The symbols between bars indicate statistical significance for Tukey post hoc pair-wise comparisons between oil injected (under restraint) and free-living animals and between CORT injected and free-living animals: * $p < 0.05$; other comparisons are not significant.

2.5 DISCUSSION

Territorial male marine iguanas showed significantly lower PHA-induced immune activity than satellite or bachelor males. At the same time, all males had indistinguishable T concentrations, but differed strongly in their baseline CORT concentrations. Linear regression models showed negative correlations between baseline CORT concentrations and immune activity and between baseline CORT and body condition. We confirmed this apparent role of CORT in immunosuppression by experimentally elevating CORT in males and detecting a decrease in immune activity. Our result is overall consistent with the idea of a resource-based trade-off between the immune system and reproduction (Rigby & Moret 2000; Sheldon & Verhulst 1996), but we could not find an involvement of T in this trade-off as suggested by Folstad and Karter (1992).

2.5.1 Body condition and CORT

Glucocorticoids are often elevated during starvation (Kitaysky et al. 1999; Romero & Wikelski 2001). One of the general physiological roles of glucocorticoids is to help supply adequate amounts of energy during strenuous times (McEwen & Wingfield 2003; Moore et al. 2000; Romero 2002; Wingfield et al. 1998). In our study, territorial males had low body condition but high baseline CORT, suggesting that these males were faced with strong energy demands during their vigorous reproductive activities. In fact, Trillmich (1983) found that territorial male marine iguanas shorten their foraging time and even fast for some time (median of fasting period: 12 days) to ensure that they keep their territories. They lose up to 26% body weight during this period, which correlates strongly with the length of their fasting period (Trillmich 1983). (Wikelski et al. 1996) found one territorial male that fasted for 35 days, i.e., did not feed during the entire reproductive period.

Hematocrit is often positively correlated with body condition (Amand 1986; Cucco et al. 2002; Potti et al. 1999). Surprisingly however, territorial iguanas showed the opposite pattern: high hematocrit but low body condition, as well as a negative correlation between immune activity and hematocrit. Two explanations for elevated hematocrit in territorials are possible. First, high hematocrit may be a prerequisite for high aerobic capacity to satisfy the higher oxygen requirements of reproductive behavior. For example, jungle fowls with higher hematocrit had higher aerobic capacity (Chappell et al. 1997). Second, an elevated hematocrit might indicate dehydration (Fitzsimons & Kaufman 1977). During hibernation, desert reptiles (*Varanus griseus*) increase their hematocrit because they do not eat or drink, resulting in a state of dehydration (Haggag et al. 1974).

2.5.2 T and immune reactivity

Contrary to numerous studies in vertebrates, which have found elevated T concentrations at the time when immune activity is reduced (Hillgarth & Wingfield 1997; Nelson & Klein 2000; Verhulst et al. 1999; Zuk et al. 1995), T was not elevated when the PHA response was suppressed in our territorial

males. This suggests that T plays a less important role as immunosuppressive agent in reproductive male marine iguanas than postulated in the immunocompetence handicap hypothesis. We did not test, however, whether the immune system was suppressed as a consequence of elevated T at an earlier point during reproduction, such as when T concentrations were elevated during territory establishment. Our results could also be expected if the immune system of territorials is more sensitive against circulating T than in other male groups. Thus, T could have caused immunosuppression in territorial males, although T concentrations were statistically equivalent in all male groups. Furthermore, it might be possible that steroid binding protein concentrations decreased in territorial males while measured T concentrations were not different between male groups. This could result in territorial males having higher levels of free T than the other male groups as shown for CORT in tree lizards *(Urosaurus ornatus)* (Jennings et al. 2000). Overall, we could not demonstrate a direct effect of T on the immune system in male marine iguanas, but an experimental verification of this result remains to be done.

2.5.3 CORT and immune reactivity

Previous studies have shown that glucocorticoids have bi-directional effects on immune function, such that temporarily elevated plasma concentrations of glucocorticoids may be immune enhancing, whereas chronically elevated concentrations may be immunosuppressive (Dhabhar & McEwen 1997). We assume, based on previous findings (Nelson 2003), that our territorial males had elevated baseline CORT concentrations over several weeks to satisfy the high energy demands associated with continuous vigorous reproductive behavior (cf. Greenberg & Wingfield 1987; Guillete et al. 1995). Therefore, chronically elevated CORT concentrations can have a suppressing effect on immune activity in territorials as we found in our study. Such immunosuppression could be adaptive because CORT may reallocate scarce nutritional reserves away from costly immune responses during situations in which an animal is under stress (Apanius 1998).

In our experiment, the PHA response was reduced in both CORT injected animals and restrained animals as compared to free-ranging animals. This confirms our result of CORT-induced immunosuppression in territorial males and indicates that both exogenous and endogenous CORT concentrations can cause immunosuppression in marine iguanas. Interestingly, the PHA response was similar when CORT was elevated by injection or by restraint, although CORT concentrations were significantly higher in CORT injected than in restraint animals. We therefore hypothesize that CORT concentrations surpassed a threshold that caused immunosuppression, while above the threshold the amount of CORT did not suppress immune response any further.

2.5.4 CORT and T interactions

Territorial males had the highest circulating baseline CORT concentrations in our study, possibly to provide sufficient energy for their intense aggressive behavior (Goymann & Wingfield 2004; Moore & Jessop 2003; Wingfield et al. 1998). However, CORT is typically highest during breeding in many

species for reasons that are currently unknown (Romero 2002). In contrast, all males had similar circulating T concentrations as we had expected based on previous findings. This suggests that T in territorial marine iguanas is high only during the establishment of territories and the early reproductive period; during the peak of reproduction, it does no longer differ from other marine iguana phenotypes (Nelson 2003).

Such a variability in T concentrations during reproduction is somewhat expected from the challenge hypothesis (Wingfield et al. 1990) which states that T is highest during times of social instability e.g. during territory establishment. Similarly, Manzo et al. (1994) have shown in lizards *(Podarcis sicula sicula)* that T concentrations are elevated during the territorial establishment and defense period, but low during the mating phase. CORT on the other hand followed opposite trends. Manzo et al. (1994) suggested that CORT, which is released by the adrenals after territories are established, acts on T synthesis to reduce aggressive behavior and thereby allows breeding. In fact, CORT and T have been found to be antagonistic in many reptiles (Grassman & Hess 1992; Moore et al. 1991; Tokarz 1987). A variation of the Energetics-Hormone Vocalization (EHV) Model proposed for anuran calling behavior by Emerson (2001) may also explain the negative association between T and CORT concentrations in male marine iguanas: T increases reproductive behavior, but due to high energetic demands of reproductive behavior, plasma CORT concentrations also increase. At some point, CORT concentrations surpass a threshold and create a negative feedback for T concentrations, which then decline.

So far it is unclear whether reproductive behavior, glucocorticoids and T interact in the same way in reproductive males of different vertebrate species. Glucocorticoid concentrations have been found to differ in males depending on their reproductive state or rank in a social hierarchy. Beletzky et al. (1989) found in red-winged blackbirds *(Agelaius phoeniceus)* that territory owners had higher CORT concentrations than adult floaters presumably because of greater energetic demands. Greenberg et al. (1984) measured lower CORT concentrations in dominant lizard males *(Anolis carolinensis)* than in subordinates, contrary to what is often shown in other taxa.

Such variability in glucocorticoid concentrations may be explained by the amount of energetic costs needed to acquire and maintain territorial or dominance status (Goymann & Wingfield 2004). Accordingly, we hypothesize that energetic costs and concomitantly plasma CORT are high in territorial male marine iguanas because their territoriality has to be defended and can only be maintained with constant physical aggression toward rivals. On the other hand, in tree lizard males *(Urosaurus ornatus)*, territorials and non-territorials had similar basal concentrations of total plasma CORT, but territorial males showed a greater androgen-glucocorticoid-binding globulin capacity than non-territorial males (Jennings et al. 2000). This could have resulted in higher concentrations of free CORT in non-territorial versus territorial males (Jennings et al. 2000). It remains unclear whether CORT binding globulins (CBGs) might also differ among male marine iguana types in addition to the differences we found in circulating plasma concentrations of CORT (Breuner & Orchinik 2001).

In general our data support the expected trade-off between reproductive activity and immune responses. This trade-off is most likely mediated by the fact that both the expression of sexual characters and mounting an immune defense require significant amounts of energy or nutrients (Demas et al. 1997; Gustafsson et al. 1995; Hamilton & Zuk 1982; Hanssen et al. 2004; Lochmiller & Deerenberg 2000; Martin et al. 2003; Råberg et al. 2000; Sheldon & Verhulst 1996). It is also possible, however, that hormones such as T and CORT may play a role in the avoidance of auto-immune disease (Råberg et al. 1998) or help to influence how immune resources are allocated, as formalized by the immunoredistribution hypothesis (Bilbo et al. 2002; Braude et al. 1999; Dhabhar et al. 1995). Further, risk of infection, as mediated by steroid hormones, may affect immune-endocrine trade-offs, but little empirical work has been done to test this hypothesis.

Despite this multitude of factors that can theoretically be involved, our data demonstrate a strong pattern suggesting that CORT concentration is an important factor driving differences in immune function among groups of male marine iguanas. We hypothesize that other factors, such as hematocrit and body condition, probably influence immune function as well, but most likely by indirect routes, which are ultimately mediated by CORT. Indeed, CORT and body condition are linked to each other and, in turn, influence reproductive behavior. We propose that CORT has an important integrative role in modulating marine iguana behavior and physiology during the strenuous reproductive period. Most likely, elevated CORT concentrations in reproductively active males coordinate the immune system and reproductive activities in order to utilize the available energy as efficiently as possible.

2.6 ACKNOWLEDGEMENTS

2.7 REFERENCES

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Fig. 1 Territorial male marine iguana during reproduction with elaborated ornamentations such as elongated spines and green coloration. They frequently show ritualized head-bobbing, a typical reproductive behavior to attract females and threaten off rivals. Territorial males, which defend clustered mating territories, are often surrounded by numerous females, and receive 95% of all copulations.

Fig. 2 Group of bachelor male marine iguanas during the reproductive season. These are mostly males that were territorial in previous reproductive seasons, but are reproductively quiescent during the current year. They do not exhibit reproductive behavior nor ornamentation and accompany other bachelors outside the clusters of reproducing males.

Fig. 3 A territorial fight between two male marine iguanas. It includes vigorous head bobbing display and males push each other by locking their head with the triangular protrusions on the top. Sometimes they bite during a fight.

Fig. 4 Phytohemagglutinin (PHA) injection to investigate immune activity. PHA causes local swelling and edema, driven by mitogenesis and infiltration of immune cells into injected tissue.

Fig. 5 Measurement of the maximum swelling of the toe-web before and 18 hours after PHA injection using a pressure sensitive spessimeter from Teclock, model SI-510.

Chapter 3

Rapid phenotype transition
within a reproductive season in male marine iguanas –
a matter of hormones and the environment?

Silke Berger, Martin Wikelski,
Elisabeth K.V. Kalko, and Thomas Rödl

3.1 ABSTRACT

Males of numerous vertebrate species exhibit distinct reproductive phenotypes. In some species, reversible changes in phenotype occur from one reproductive season to another. According to the relative plasticity hypothesis, different phenotypes, like territorial, satellite or bachelor males, are regulated by hormones. In the marine iguana, males have been observed to rapidly change phenotype even within the same reproductive season. Here, we report on the frequency and endocrine correlates of rapid phenotype transitions in marine iguanas. We compared testosterone (T) and corticosterone (CORT) concentrations between males that switched phenotype and others that maintained their phenotype during the reproductive season. A non-reproductive male group, bachelors, acted as a control in our study to detect potential environmental correlates. We found that T concentrations of males that switched from a less to a more aggressive phenotype, for instance from satellite to territorial, remained high, while they decreased in all other males. In contrast, CORT concentrations increased while body condition indices decreased in both reproductive and non-reproductive male phenotypes during the study period, indicating environmental effects. In conclusion, T appears to be important during the early phase of territorial establishment and capacitates males to participate in reproduction. Which strategy, however, is realized by a male at what time is probably a rather flexible decision on the individual level. T contributes to the establishment of the respective phenotype and depends on individual condition and environmental influences. Extension of phenotype switches far into the reproductive season may be an adaptation to the unpredictable climate on the Galápagos Islands.

3.2 INTRODUCTION

Males of various vertebrate species show annual flexibility in reproductive phenotype and change between alternative strategies, such as territorial and non-territoral, depending on environmental conditions (Beletsky et al. 1989; Gross 1996; Knapp et al. 2003; Mendonca et al. 1985; Moore 1991; Wikelski et al. 2004). In contrast, males of species with fixed reproductive phenotypes assume one phenotype at or before sexual maturity and do not change it during their adult lives (Mason & Crews 1985; Moore 1991; Moore & Thompson 1990).

Overall, reproductive phenotypes mostly differ in morphology, i.e. ornaments and color, and/or behavior (Beletsky et al. 1989; Gross 1984; Gross 1996; Howard 1978; Moore et al. 1998; Moore & Thompson 1990). A well-documented example is the bluegill sunfish with three types of males (Gross 1979; Gross 1984). Large males are brightly colored and territorial, medium sized males mimic females in color, and small males are cryptic sneakers.

According to the relative plasticity hypothesis, differences in morphology and behavior are fostered by hormones during early development in fixed systems, whereas activational influences of hormones during adult lives are rather important in plastic systems (Moore 1991). Thus, males with plastic phenotypes differ in their adult hormone profiles, while males with fixed alternative phenotypes have identical hormone profiles (Moore 1991). Although there is no consistent evidence that plastic phenotypes are regulated by testosterone (T) (Brantley et al. 1993; Moore & Thompson 1990), support for hormonally mediated phenotypic transition is provided by studies where males have been treated with androgens. In Azorean rock-pool blennies *(Parablennius sanguinolentus parvicornis)* satellite males developed secondary sex traits such as longer and wider genital papilla and anal glands with androgen treatment. The traits were less expressed or absent in satellite males without treatment (Oliveira et al. 2001).

In our study animal, the marine iguana, males exhibit either a territorial, satellite or sneaker phenotype, or they skip reproduction and behave as reproductively inactive bachelors (Carpenter 1966; Eibl-Eibesfeldt 1966; Wikelski et al. 1996). They change phenotype from one reproductive season to the next depending on environmental conditions (Carpenter 1966; Eibl-Eibesfeldt 1966; Wikelski & Baeurle 1996). However, some male marine iguanas may exhibit phenotypic flexibility even within a reproductive season and switch from a less to a more aggressive phenotype or vice versa (Wikelski pers. comment). Rapid phenotype transitions during reproduction are rare in vertebrates compared to cases of inter-annual flexibility, except in some group-breeding anurans like spring peepers *(Pseudacris crucifer)* that can switch back and forth between caller and satellite roles, depending on the pressure of competition between males (Lance & Wells 1993).

While annual flexibility of male marine iguanas is well described, nothing is known about the phenomenon of rapid phenotype transition within a season – how often it occurs in a population, its function and the proximate mechanism underlying the observed switch. It has been shown previously that T concentrations differ between reproductive phenotypes in marine iguanas (Moore et al. 2000;

Wikelski et al. 2004). T treatment in male marine iguanas induced a phenotype switch, giving further evidence that T controls the expression of distinct phenotypes in this species (Wikelski et al. 2004). Therefore, the question arises whether rapid phenotype transitions within a season are also regulated by changes in T. In other species, T concentrations of individuals that do not participate in reproduction from the beginning on are presumably low, whereas T increases when individuals suddenly adopt a reproductive phenotype (Greenberg & Crews 1983; Tokarz 1995).

In our study, we compared T concentrations of male marine iguanas that either switched between a territorial, satellite or bachelor phenotype, or that maintained the same phenotype throughout the reproductive season. The animals were measured twice, at the time of territorial establishment before a potential phenotype transition and later during the mating period after phenotype transition of the respective males took place.

According to the theory about the activating influence of T, we formulated the following hypotheses: (1) T concentrations should increase in males that switch during the reproductive period from a non-reproductive phenotype (bachelor) or a less aggressive reproductive phenotype (satellite) to a more aggressive phenotype (territorial or satellite, respectively), while (2) T concentration should decrease in males that stop to participate in reproduction and switch to a non-reproductive bachelor phenotype or give up territories and adopt a satellite strategy. (3) In contrast, T concentrations in males that maintain a territorial phenotype throughout reproduction should remain high or even increase until mating, because male marine iguanas usually become more aggressive over the course of the reproductive period, expressed in an increase in the number of fights (Partecke et al. 2002). (4) As bachelor males are reproductively inactive, T concentration should be low throughout the reproductive season.

Since other hormones, in particular glucocorticoids, respond to a variety of environmental and social stressors (Knapp & Moore 1996; Moore & Thompson 1990; Wingfield et al. 1998), we also measured corticosterone (CORT) concentrations, the principal stress hormone in reptiles, to detect possible correlations between CORT and reproductive behavior or environmental changes (Greenberg & Wingfield 1987; Romero 2002; Romero & Wikelski 2001). Additionally, we determined body condition of all males. We took bachelor males as a control group in our study because they do not engage in reproduction. If non-reproductive bachelor males show the same pattern of CORT concentrations and body condition as reproductively active males, then environmental effects could have caused the pattern rather then reproductive activity per se. If the pattern differs between non-reproductive and reproductive males, we would conclude that reproductive behavior caused the difference.

3.3 METHODS

3.3.1 Study site and animals

Our study site was located at the south eastern coast of the uninhabited Galápagos Island Santa Fé at Miedo (0°50'S, 90°02'W) where marine iguanas live in large aggregations of about 1500 animals per

1 km coastline. Males establish small territories on the lava rocks above the inter-tidal zone during the reproductive period.

We studied male marine iguanas during territorial establishment from November 2 to 9, 2003 and at the peak of mating from December 14 to 18, 2003. Animals were caught with a bamboo pole with a noose and marked with synthetic paint. The paint remained throughout the study period. A previous study has shown that paint markings did not influence iguana behavior (Audet & Wikelski, unpublished data).

3.3.2 Flexible reproductive phenotypes in male marine iguanas

Male marine iguanas with distinct reproductive phenotypes differ in morphology and behavior. Territorial males are large and visually most conspicuous with red and green skin coloration and elongated dorsal spines. They defend clustered mating territories by patrolling and by ritualized head-bobbing to attract females and threaten off rivals (Carpenter 1966; Wikelski et al. 2001). Territorial males have the highest reproductive success and show the highest steroid hormone concentrations of all phenotypes (Berger et al. 2005; Moore et al. 2000; Wikelski et al. 2004).

Satellite males are smaller and less ornamented than territorial males. They do not defend territories but roam around and grab females that leave the territories of males for foraging. Bachelor males remain reproductively inactive during a reproductive season but often have been territorial in previous years (Dellinger 1991). Bachelors stay peacefully together with other bachelors outside clusters of reproductively active males (Dellinger 1991; Wikelski et al. 1996).

3.3.3 Categorization of male reproductive phenotypes

The study males were randomly chosen from the overall population after a first discrimination between reproducing and non-reproducing males by morphology. Observational categorizations between territorial, satellite and bachelor males were performed by 10 min observations prior to first capture during times of territorial establishment. After handling, blood sampling and release, each male was observed at least 5 times for 10 min at different times during the period of territorial establishment to confirm the phenotype. A male was categorized as territorial if it showed vigorous head-bobbing against rivals, exhibited typical morphological traits such as green coloration, elongated spines and a broad head, and occupied a specific area for more than 5 consecutive days (Wikelski et al. 1996). One month later, at the peak of mating, males were again observed several times prior to the second capture to detect possible phenotype transitions.

We defined 4 groups of 37 males: (1) males that switched from a less active/inactive to a more active and thereby more aggressive strategy, including a) from bachelor to satellite (n = 2), b) from bachelor to territorial (n = 1), and c) from satellite to territorial (n = 7); (2) males that switched from a more active and aggressive to a less active/inactive strategy, including a) from satellite to bachelor (n = 1), b) from territorial to bachelor (n = 3), and c) from territorial to satellite (n = 2), and finally, males

that did not change phenotype, i.e. (3) bachelor (n = 7) and (4) territorial males (n = 14). Altogether, we marked 50 males at the beginning of the mating season and recaptured 37 males for comparison during the peak of mating.

3.3.4 Hormones and body condition

Because hormone concentrations in marine iguanas are influenced by an endogenous circadian rhythm, entrained by photoperiod, food intake, and tidal cycle (Woodley et al. 2000), we sampled from noon to late afternoon over 8 consecutive days during high tide, where marine iguanas bask on lava rocks to digest (Wikelski & Hau 1995). We applied a standard capture and restraint stress protocol to measure basal and induced plasma CORT concentrations (Romero & Wikelski 2001; Wingfield et al. 1994).

After observational phenotype categorization, the respective male was caught and an initial blood sample of about 1 ml was collected with a 22 G needle into a sodium heparinized Vacutainer tube within 3 min of capture to get the CORT baseline concentrations (Becton Dickinson, Franklin Lakes, NJ). We measured body temperature in the cloaca with a digital thermometer (accuracy 0.1°C) immediately after the initial blood collection to control for potential influences of body temperature on CORT concentrations (Romero unpublished data) and excluded animals with body temperatures lower than 30°C. To quantify the CORT concentration in relation to acute stress, individual iguanas were subsequently held in an opaque cloth bag and a second blood sample was collected after 30 min (CORT response concentration to restraint stress in a bag).

Blood samples were stored for up to 6 hours in a thermos bottle on cooling gel packets and then centrifuged (Baacklab Microcentrifuge, 6 x 1.5 ml, 6000 min -1, ran by a 12 V car battery) at 2000 g for 5 minutes. Plasma was removed from each sample and an aliquot of the plasma of the initial blood samples was separated for T analysis. Plasma vials were stored at 4°C for 7 days in a gas-fueled cooling box until they could be frozen at -20°C. After collecting the second blood sample, each iguana was weighed to the nearest 10 g and the snout-vent length (SVL) was determined to calculate a body condition index ((body mass/SVL^3)*10^6) as a measure of the physical condition of the animal (Laurie 1989). SVL did not differ significantly between males of distinct phenotypes ($F_{2,47}$ = 0.965, p = 0.388).

All procedures on iguanas were performed in accordance with the guidelines set forth by the American Society of Ichthyologists and Herpetologists. They were approved by the Princeton Institutional Animal Care and Use Committees in accordance with NIH standards for the use of animals in research and conducted with permission of the Galápagos National Park Service, Republic of Ecuador.

3.3.5 Radioimmunoassays and statistical analysis

CORT and T concentrations were analyzed by indirect radioimmunoassay as described in Wingfield et al. (1992). A small amount of tritium-labeled steroid was added to determine subsequent recovery, and

then the steroids were extracted with dichloromethane. T antibody, T 3003, from Wien Laboratories, Succasunna, NJ, and CORT antibody, B3-163, from Esoterix Endocrinology, Austin, TX, were used. Each sample was assayed in duplicate. Intra-assay/inter-assay variation of the hormone assays was on average 5.6/22.3 % for T and 3.8/18.8 % for CORT. Assay sensitivity was at 1.05 ng/ml for T and 0.92 ng/ml for CORT. All samples were above the detection limit.

Data were analyzed with SPSS 10.0 for Windows. Testosterone concentrations were $\log_{10}$ transformed to achieve normal distribution. We used repeated-measures general linear models (RM-GLM) to test within- and between-subject and interaction effects in an overall model. α was set at p = 0.05. Additionally, we used single paired t tests to compare hormone concentrations and body condition of males within a phenotype between times of territorial establishment and mating. We applied single unpaired t tests to separately determine differences in hormone concentrations and body condition between distinct phenotypes during territorial establishment and mating. We applied Bonferroni corrections for multiple comparisons ($\alpha' = \alpha/k = 0.05/2$). Here α' was set to p = 0.025.

3.4 RESULTS

3.4.1 Males with phenotype switch

Almost half (43 %) of the study males (total n = 37) switched reproductive phenotype during reproduction. 62.5 % of those males switched from a less to a more active phenotype, while 37.5 % switched from a more active to a less active phenotype.

We found a significant general decrease in T from times of territorial establishment to mating over the course of the reproductive season. However, the extent of T changes differed significantly between both groups (RM-GLM, time: $F_{1,14} = 16.152$, p = 0.001, time × type of switch: $F_{1,14} = 7.863$, p = 0.014, Tab. 1). Only males that switched from a more active to a less active phenotype showed a significant decrease in T concentrations (t = 4.748, p = 0.005, Fig. 1 A), while T concentrations in males that switched from a less to a more active phenotype remained high throughout reproduction (t = 0.947, p = 0.368, Fig. 1 A). This resulted in a significant difference in T between the two male groups at mating time (t = 2.86, p = 0.013, Fig. 1 A). In contrast, T concentrations did not differ between groups during the time of territorial establishment (t = -0.902, p = 0.382, Fig. 1 A).

Overall, CORT baseline concentrations increased in males during the season (RM-GLM, time: $F_{1,14} = 5.194$, p = 0.039, Tab. 1). However, CORT baseline concentrations tended to increase in separate paired t tests only in males that switched from a less to a more active phenotype (switch to more active phenotype: t = -2.308, p = 0.046; switch to less active phenotype: t = -1.443, p = 0.209). Body condition indices decreased during the season in an overall model (RM-GLM, time: $F_{1,14} = 51.130$, p < 0.001, Tab. 1) as well as in both male groups when tested separately (switch to more active phenotype: t = 6.696, p < 0.001; switch to less active phenotype: t = 3.784, p = 0.013). CORT baseline concentra-

tions and body condition indices did not differ between males of opposite phenotype switches, neither during territorial establishment (CORT baseline: t = -0.657, p = 0.522, Fig. 1 C; body condition index: t = -0.655, p = 0.523, Fig. 1 G) nor at mating time (CORT baseline: t = 0.826, p = 0.422, Fig. 1 C; body condition index: t = -0.664, p = 0.528, Fig. 1 G).

In contrast to CORT baseline concentrations, CORT response concentrations did not significantly change after acute stress over the course of the reproductive season (RM-GLM, time: $F_{1,14}$ = 2.587, p = 0.13, Tab. 1). However, CORT response concentrations tended to increase slightly in males that switched from a more to a less active phenotype (t = -2.776, p = 0.039, Fig. 1 E). There were no significant group differences in CORT response concentrations at both times of territorial establishment and mating (territorial establishment: t = 0.345, p = 0.522; mating: t = 0.826, p = 0.422, Fig. 1 E).

	Testosterone		CORT baseline		CORT response		body condition	
with tactic switch	F	p	F	p	F	p	F	p
time	16.152	**0.001**	5.194	**0.039**	2.587	0.130	51.130	**<0.001**
type of switch	2.422	0.142	0.190	0.669	0.384	0.545	0.572	0.462
time * type of switch	7.863	**0.014**	1.130	0.306	0.085	0.775	0.187	0.672
without tactic switch								
time	12.997	**0.002**	17.981	**<0.001**	6.091	**0.023**	139.832	**<0.001**
phenotype	1.284	0.271	0.047	0.836	0.129	0.723	3.309	0.085
time * phenotype	0.001	0.973	0.000	0.993	0.232	0.635	4.009	0.06

Tab. 1 Changes in testosterone, CORT baseline, CORT response concentrations, and body condition index of male marine iguanas over the course of the reproductive period, i.e. between times of territorial establishment and peak of mating (within-subject factor "time"), and between types of phenotype switch (between-subject factor "type of switch": Males that switched from a less to a more active phenotype (n = 10) and vice versa (n = 6)) or between reproductive phenotypes (between-subject factor "phenotype": Bachelor (n = 7) or territorial (n = 14)). Repeated-measures general linear models (RM-GLM) were used. p < 0.05.

3.4.2 Males with constant reproductive phenotype

In our study, 38 % of the males maintained their territorial phenotype from times of territorial establishment until mating, while only 19 % remained reproductively inactive bachelor males. In an overall model of T profiles, T concentrations decreased in both male groups over the course of the reproductive season (RM-GLM, time: $F_{1,19}$ = 12.997, p = 0.002, Tab. 1). While T concentrations of bachelor
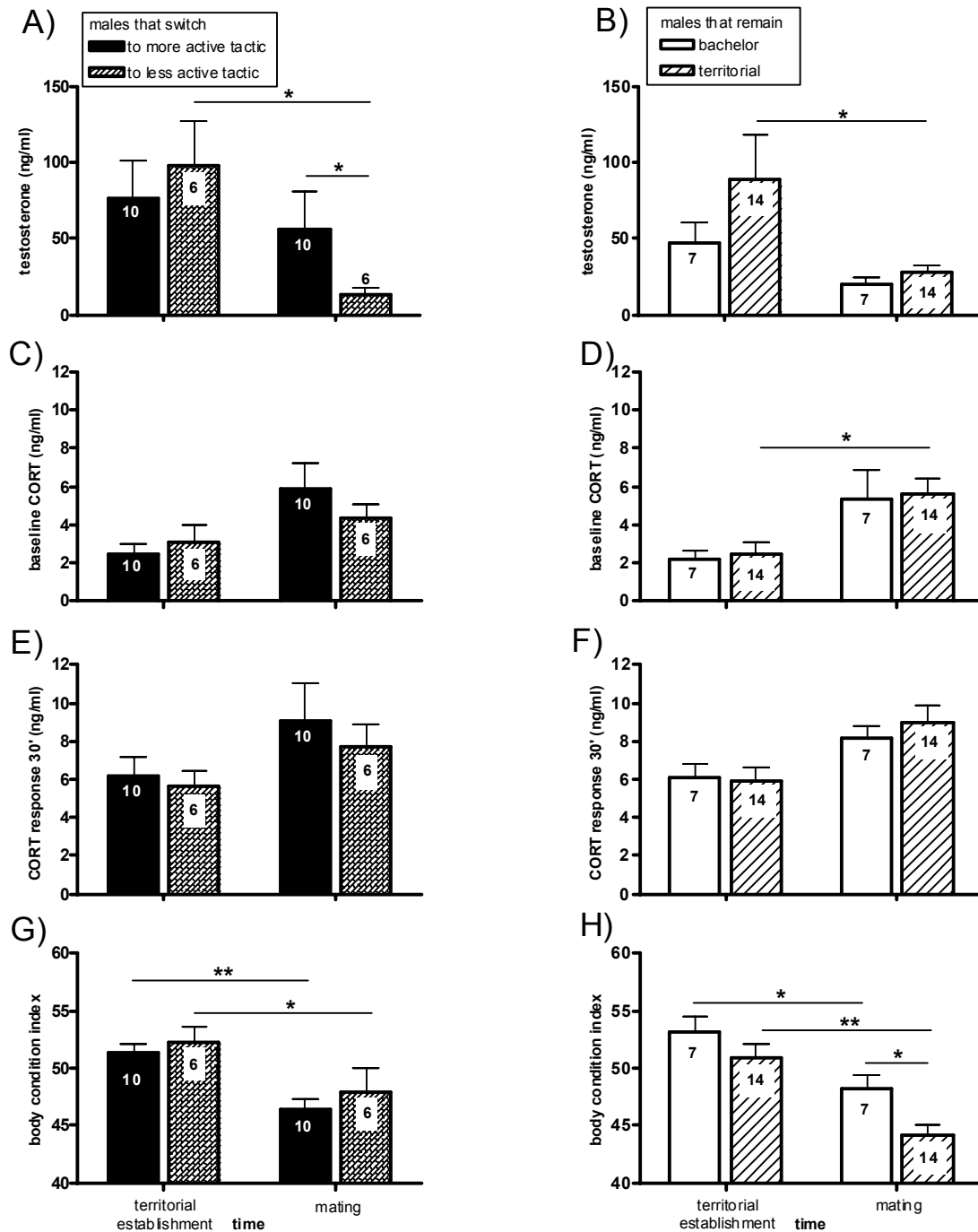
Fig. 1 Testosterone, baseline CORT, CORT response after 30 min of acute stress (restraint in a bag), and body condition index of male marine iguanas that switched reproductive phenotype (**A, C, E, G)** (to more active: n = 2 from bachelor to satellite, n = 1 from bachelor to territorial, n = 7 from satellite to territorial; to less active: n = 1 from satellite to bachelor, n = 3 from territorial to bachelor, n = 2 from territorial to satellite) or maintained their reproductive phenotype (bachelor or territorial) from time of territorial establishment until the peak of mating season **(B, D, F, H)**. Paired and unpaired t tests were used for analysis. Mean values ± 1SE are presented. ** P < 0.001; * P < 0.025 after Bonferroni correction.

males decreased only slightly (t = 2.198, p = 0.07, Fig. 1 B), T decreased significantly in territorial males (t = 3.113, p = 0.008, Fig. 1 B). There were no differences in T between bachelors and territorial males at both times of territorial establishment and mating (territorial establishment: t = -1.201, p = 0.241; mating: t = -1.087, p = 0.291, Fig. 1 B).

CORT baseline and CORT response concentrations increased during reproduction in all males independent of phenotype (RM-GLM, time: CORT baseline: $F_{1,19}$ = 17.981, p < 0.001; CORT response: $F_{1,19}$ = 6.091, p = 0.023 Tab. 1). However, the increase in CORT baseline concentration was only significant in territorial males (territorials: CORT baseline: t = -3.785, p = 0.002, CORT response: t = -2.256, p = 0.042; bachelors: CORT baseline: t = -2.45, p = 0.05, CORT response: t = -1.954, p = 0.099). CORT baseline and CORT response concentrations of territorial and bachelor males were similarly high both at times of territorial establishment (CORT baseline: t = 0.737, p = 0.468; CORT response: t = -0.156, p = 0.878 Fig. 1 D) and mating (CORT baseline: t = 0.662, p = 0.514; CORT response: t = -0.553, p = 0.587, Fig. 1 F).

Males of both phenotypes showed decreasing body condition indices in the overall model (RM-GLM, time: $F_{1,19}$ = 139.832, p < 0.001, Tab. 1) as well as in single paired t test analyses (territorials: t = 11.831, p < 0.001; bachelors: t = 6.181, p = 0.001, Fig. 1 H). During territorial establishment, males of the two phenotypes did not differ in body condition (t = 0.019, p = 0.985). However, at times of mating, territorial males showed significantly lower body condition indices than bachelors (t = 2.631, p = 0.016, Fig. 1 H).

To summarize, T concentrations remained high over the course of the reproductive season in male marine iguanas that switched from a less to a more active tactic. In contrast, T concentrations of all other males decreased. Males showed increasing CORT concentrations independent of reproductive phenotype and, concomitantly, decreasing body condition indices during the reproductive season. However, the most active and aggressive males, the territorials, showed the most pronounced CORT increase during reproduction and had the lowest body condition at times of mating.

3.5 DISCUSSION

3.5.1 Testosterone and reproductive behavior

Almost every second male of our study animals switched reproductive phenotype, which was much more than we expected according to previous studies (Partecke et al. 2002; Wikelski et al. 2004). Our results demonstrate further that changes in T concentrations over the course of reproduction (T profiles) differed significantly depending on the direction of phenotype switch. T concentrations decreased when males switched from a more to a less active phenotype, which is consistent with our expectations. However, contrary to our expectations, T concentrations did not increase but remained at high initial levels over the course of reproductive season when males switched from a less to a more active phenotype. This indicates that the initial breeding concentration of T at the beginning of repro-

duction was probably sufficient to activate reproductive behavior, but T appeared not to be directly linked to the amount of reproductive activity over the course of reproduction.

Furthermore, we expected increasing or at least constant T concentrations in males that maintained the territorial phenotype throughout reproduction, because the frequency of fights in marine iguanas usually increases during the reproductive season and reaches a maximum at the peak of mating (Partecke et al. 2002). The territorials, however, showed decreasing T concentrations over the course of our study. This result was unexpected, since the intensity of aggressive territorial and mate guarding behavior is often directly associated with the circulating level of T in other species (Wingfield et al. 1990; Wingfield & Marler 1988). Furthermore, males of various species sustain strenuous activities by keeping their T concentration high probably not to loose territorial space (Clutton-Brock et al. 1992; Cooper & Vitt 1993; Westcott 1997). Indeed, it has been shown in lizards *(Podarcis sicula sicula)* that T concentrations are elevated during the territorial establishment and defense period (Manzo et al. 1994). However, in our study, T was low during the mating phase, which may reduce inter-sexual aggressive behavior and, thus, facilitates mating as shown in lizards *(Podarcis sicula sicula)* (Manzo et al. 1994).

Surprisingly, bachelor male marine iguanas showed a tendency of higher T concentrations during the early season as compared to the late season, although they remain reproductively inactive throughout the whole reproductive season. We therefore suggest that all males, independent from their initial phenotype, are hormonally prepared to engage in territorial and reproductive activities early in the season. While we could document a seasonal decrease in the concentrations of T in almost all males except in males that switched from a less to a more active phenotype, in which case T concentrations remained high, they neither differed significantly between territorial and bachelor males during territorial establishment nor during the mating phase. We expected higher T concentrations in territorial than in bachelor males according to previous studies, which demonstrated significantly higher T concentrations in more reproductively active phenotypes than in less active or inactive phenotypes (Moore et al. 2000; Wikelski et al. 2004). The reason for the lack of T differences between phenotypes in our study is unknown, but perhaps, the sample sizes were too low to detect such differences.

Overall, the results indicate that elevated T concentrations are probably not directly correlated with the amount of aggressive reproductive behavior per se in marine iguanas, but are affected mainly by season. T concentrations were high at the beginning of the season during territorial establishment, a result which has been also shown for other vertebrate species (Moore 1988; Wingfield & Ramenofsky 1987; Wingfield 1985; Wingfield & Moore 1987). Further evidence of the importance of T early in the season is supplied by prolonged high concentrations of T in male marine iguanas that switched from a less to a more active phenotype and thus were in an extended period of territorial establishment. It is unknown for marine iguanas, whether the sustained elevated T concentrations result from male-male interactions as stated by the challenge hypothesis, which proposes T releases in conjunction with be-

havioral interactions (Wingfield et al. 1990). However, T may allow for phenotype transition of male marine iguanas that participate later in reproduction either as a territory owner or as a roaming satellite male.

Ultimately, since T appears not to be directly linked with the quantity of aggressive reproductive behavior, the question arises whether the intensity of aggressive interactions in male marine iguanas is modified by other hormones such as CORT. In a previous study, only male marine iguanas that had simultaneously high CORT concentrations during experimental manipulations of the T concentrations had more territorial interactions (Wikelski et al. 2004). This indicates that CORT rather than T caused an increase in male-male interactions (Wikelski et al. 2004).

3.5.2 CORT – reproductive behavior and environmental effects

CORT, the principal stress hormone in reptiles, responds to a variety of environmental and social stressors (Knapp & Moore 1996; Moore & Thompson 1990; Wingfield et al. 1998). Its general physiological role is to supply adequate amounts of energy during strenuous times such as reproductive period, harsh climatic conditions or food scarcity (McEwen & Wingfield 2003; Romero 2002; Romero & Wikelski 2001). Therefore, we expected elevated CORT concentrations in males of more active and aggressive reproductive phenotypes as higher levels of aggressiveness usually require higher amounts of energy according to the energy mobilization hypothesis (Romero 2002). This assumption has been confirmed by previous studies, where territorial male marine iguanas had the highest CORT baseline concentrations compared to other phenotypes (Berger et al. 2005; Wikelski et al. 2004). However, bachelors and territorials, or males that switched phenotype, respectively, did not differ in CORT baseline concentrations in the present study. Our results demonstrate, instead, that CORT baseline concentrations tended to increase during reproduction in all reproductively active males as well as in inactive bachelors. This indicates that environmental effects, such as climatic changes or food availability, rather than distinct reproductive strategies per se influence CORT concentrations.

Abiotic effects on CORT concentrations have been detected in various species of reptiles, e.g. male tree lizards *(Urosaurus ornatus)*, showed elevated CORT concentrations in harsher, drier years than in favorable, wetter years (Knapp et al. 2003). Furthermore, western fence lizards *(Sceloporus occidentalis)* responded with increasing CORT concentrations to food scarcity (Dunlap & Wingfield 1995). All males in our study showed decreasing body condition independent of phenotype, which further supports the possible effect of abiotic environmental variables, such as the decline of food algae density with increasing sea temperatures during the reproductive period. Since CORT helps to metabolize energy resources, the reduced body condition indices later during reproduction can be related to the simultaneously elevated CORT baseline concentrations.

Territorial males showed the most pronounced CORT increase during reproduction and had the lowest body condition indices of all males. This was probably caused by abiotic factors and reproductive activities. The low body condition and high CORT levels may be related to at least two

aspects. Firstly, it is known that territorial males shorten their foraging time and even fast for some time to ensure that they keep their territories (Trillmich 1983). Furthermore, territorials are more frequently involved in fights because they do not leave their territories compared to satellites that may leave the area if social interactions become too stressful (Wingfield et al. 1998).

Territorial males and males that switched from a more to a less active phenotype showed a tendency of increasing CORT response concentrations to acute stress (restraint in a bag) over the course of the reproductive season. Elevated adrenal responsiveness may be associated with behavioral changes during reproduction and can be advantageous at times of higher frequency of aggressive interactions (Wingfield 1994). In general, modifications in adrenal responsiveness may allow the adjustment of physiological and behavioral responses, which can help to explain the mechanism of distinct behavior of reproductive strategies (Sapolsky et al. 2000; Schwabl 1995; Wingfield et al. 1994).

3.5.3 Reasons for rapid transitions between reproductive phenotypes

From our data and from the literature we conclude that flexibility between reproductive phenotypes in marine iguanas is advantageous because of the high environmental variability of the Galápagos islands (Wikelski et al. 2004). As an example, climatic conditions during an El Niño year, where air and sea temperatures increase and concomitantly, algae growth is reduced, strongly affects the demographic structure of marine iguanas. Relative numbers and body sizes of males vary from year to year due to high mortality rates caused by El Niño years. This influences in particular large territorial males, which are more prone to starvation and related effects (Wikelski & Romero 2003). Simultaneously, those changes temporarily give smaller males a chance to behave as territorials, when the number of larger males is low.

On the Galápagos islands, seasonal changes also occur within a smaller time scale, namely during the transition between dry and wet season that overlaps with the onset of the reproductive season of male marine iguanas. During this period, air and sea surface temperatures increase significantly (+4.5°C in an average year) (Wikelski & Trillmich 1997). In our study year, there was a weak Pacific warm episode, in which the sea surface temperature was +1 C° warmer than average (NOAA ENSO Diagnostic Discussion 2003). Warming of sea water results in a reduction of algae growth and quality (Rubenstein & Wikelski 2003). All males probably lost weight because of lower food availability, resulting in lower body condition. Weak territorials were probably not longer in a position to successfully defend their territories. Abandonment of a large part of the territories subsequently leads to changes in social structures with satellites most likely taking the chance to occupy the territories. Thus, due to the unpredictability of climatic factors and thereby varying food availability within a season, it can be advantageous for some males to await the development of the current situation until a decision is achieved whether to reproduce or not. Rapid phenotype transitions therefore may occur only under particular environmental conditions. This is indicated by our result that almost half of the

study males switched phenotype within a season, whereas rapid phenotype transition was a rare event in other years, when e.g. only one out of 22 territorial males abandoned their territory (Partecke et al. 2002).

Although flexibility is generally assumed to be advantageous in variable environments, costs and limits of plasticity, like trait expression costs or choice of the wrong phenotype, have to be taken into account (Pigliucci 2005). In particular, phenotype transition involves morphological and physiological changes that require time when accompanied with longer-term reorganization of neural circuits and changes in muscle physiology (Fusani et al. 2000). Consequently, males that switch to the territorial phenotype later during reproduction may be poor quality territorials and thus may achieve lower reproductive success because of incomplete phenotype switch (Wikelski et al. 2004).

Some transitions, however, can occur very quickly such as some changes in ornamentations. Green coloration, a typical ornamentation of reproductively active male marine iguanas, is positively correlated with phenotype switch. Males that switched from a less to a more active tactic showed an increase in green coloration (Rödl et al. in prep.). Furthermore, one of the males in our study, classified as bachelor with black skin and without any signs of aggression, developed conspicuous green spots and was involved in aggressive interactions with a territorial male within a day (Rödl and Berger, pers. observation).

Overall, the reproductive system of male marine iguanas is strongly influenced by environmental changes between years as well as within a season. Hence, rapid phenotype transitions within a season probably help to maximize lifetime reproductive success similarly as transitions between years. However, whether a rapid phenotype transition within a season is carried out completely in terms of morphology and behavior and thus, life-time fitness of males that switched phenotype is the same than in males that maintained the same phenotype over the course of reproduction needs to be investigated.

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Chapter 4

Behavioral and physiological adjustments to new predators in an endemic island species, the Galápagos marine iguana

Silke Berger, Martin Wikelski, L. Michael Romero,
Elisabeth K.V. Kalko, and Thomas Rödl

4.1 ABSTRACT

Since about 5 to 15 million years, marine iguanas, endemic to the Galápagos archipelago, experience relaxed predation pressure and, presumably as a consequence, demonstrate very little anti-predator behavior. However, on some of the islands, domestic dogs and cats have been introduced during the recent past and depredate on the native iguana populations. We used this situation to investigate the degree of phenotypic plasticity in anti-predator responses in a species with an evolutionarily long history of predation relaxation and tested experimentally whether the behavior and endocrinology of marine iguanas has changed after the introduction of new predators. We compared flight initiation distances (FID) to simulated predator approach and corticosterone (CORT) concentrations in marine iguanas of different age and sex at sites with and without new predation threat. We hypothesize that FID and CORT concentrations should increase in affected populations to cope with the novel predators. Our results demonstrate that local populations of marine iguanas reacted differentially to simulated predator approach depending on whether they were previously exposed to introduced predators or not. FIDs were larger at sites with predation than at sites without predation. Furthermore, the occurrence of new predators was associated with increased stress-induced CORT levels in marine iguanas. In addition, age was a strong predictor of variation in FID and CORT levels. Juveniles, which - compared to adults - are probably more threatened by predators under natural conditions because of their smaller size, showed larger FIDs and higher CORT baseline levels as well as higher stress-induced levels than adults. The results provide evidence that naïve island species show behavioral and physiological plasticity associated with actual predation pressure, a trait that is presumably adaptive. However, the adjustments are not sufficient to cope with the novel predators. We suggest that low behavioral and physiological plasticity in the face of introduced predators may drive many island species to extinction.

4.2 INTRODUCTION

Animals on isolated islands with few or no predators, such as the Galápagos islands, typically show little escape response towards intruders in comparison with animals on the mainland (Curio 1966; Darwin 1839; Stone et al. 1994). This behavior is interpreted as the result of relaxed selection during evolutionary history because of isolation from predators (Beauchamp 2004; Blumstein et al. 2000; Maloney & McLean 1995). Consequently, the introduction of novel predators on such islands is likely to have severe consequences for the island species (Berger et al. 2001; Gittleman & Gompper 2001; Stone et al. 1994). In contrast to island species with relaxed predation, species on the mainland react with efficient escape maneuvers towards predator attacks and have been shown to quickly alter their behavior in response to an increased predation risk by existent and/or novel predators (Lima & Dill 1990; Maloney & McLean 1995). Mainland species respond with different escape tactics or behaviorally compensate for elevated vulnerability to predation. For example, juveniles and females escape quicker possibly due to their smaller body size (Shine et al. 2000; Whiting et al. 2003), and ectotherms escape earlier when slowed by low body temperature (Cooper & Vitt 2002; Rand 1964; Smith 1997).

However, it remains unclear whether and how quickly island species can retrieve or develop the ability to perceive introduced species, such as dogs and cats, as life-threatening predators and adjust their anti-predator behavior to novel threats accordingly. As an example, a comparison between the responses of experienced (mainland) and naïve (predator-free island) New Zealand robins to an introduced mammal, the stoat *(Mustela erminea)*, demonstrated that the mainland robins responded strongly to the stoat, whereas the island robins showed only a weak response (Maloney & McLean 1995). Another comparison between populations of North American ground squirrels tested the capability of predator-recognition after different periods of relaxed selection from snakes (Coss 1999). California ground squirrels *(Spermophilus beecheyi)*, which have been isolated from snakes for a time period of about 70,000 – 300,000 years, still recognize snakes as potential predators and respond to them appropriately (Coss 1999). In contrast, Arctic ground squirrels *(Spermophilus parryii)*, isolated from snakes for 3 – 5 million years, have lost the ability for snake-recognition, probably because of the longer period of relaxed selection from snakes (Coss 1999). On islands without predators, the lack of predator-recognition in native island-species due to relaxed predation and, as a consequence, their insufficient anti-predator behavior have become a conservation issue, largely because novel predators have been introduced onto many previously predator-free islands either accidentally or intentionally by humans and have driven or are driving many of the island endemics to (near) extinction (Atkinson 1989; Iverson 1978; Kruuk & Snell 1981).

Responses to a perceived threat are not restricted to the behavioral domain but also involve, and are possibly mediated by, concomitant physiological changes (Blanchard et al. 1998; Canoine et al. 2002; Selye 1950; Silverin 1998). The first flight response of a vertebrate is probably linked to epinephrine release, but due to the rapid secretion of this neurotransmitter, very few studies have ad-

dressed this mechanism in wild animals. Instead, the most commonly measured physiological indicator of responses to threats and to other environmental and social stressors is the circulating plasma glucocorticoid concentration in the blood (Moore & Thompson 1990; Sapolsky et al. 2000; Wingfield 2000; Wingfield et al. 1998). Although glucocorticoids are not directly involved in the first flight response, a higher sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis and thereby increased glucocorticoid levels can help to prepare an organism to deal better with an increased probability of predatory attacks (Romero 2002; Sapolsky et al. 2000).

We therefore investigated (1) the reaction norms of behavioral and physiological anti-predator responses in a naïve island species, and (2) whether and how fast naïve species react to the introduction of new predators. Furthermore, we address (3) whether behavioral changes are accompanied with changes in plasma glucocorticoid concentrations. In addition, we tested the influence of age and sex on anti-predator behavior and the andrenocortical responsiveness because we assumed a higher vulnerability to predation by individuals of smaller body size, in particular young animals and females (Cooper Jr. & Vitt 1985; Diaz-Uriarte 1999).

We chose the marine iguana *(Amblyrhynchus cristatus)* as our study species. Marine iguanas are endemic to the Galápagos islands and originated about 5-15 million years ago (Rassmann 1997). Galápagos is of volcanic origin and as it has never been in contact with the continent, marine iguanas evolved without predation risk by large terrestrial animal species. Marine iguanas live in huge aggregations on the rocky shores of most islands in the Galápagos archipelago. During the day, they sunbath on lava rocks to heat up before foraging in the sub- and intertidal of the cool waters that eventually arrive at Galápagos by the Humboldt Current, coming from the Antarctic (Kricher 2006). The extensive basking behavior of marine iguanas probably could only evolve in absence of serious terrestrial predators.

Since the last century, some of the Galápagos islands face a rapidly developing human population, that has reached already an estimated 25,000 inhabitants. With the people, novel predators such as domestic dogs and cats have been introduced to the islands, whose growing numbers are increasingly becoming a serious threat for most native species, including marine iguanas (Cayot et al. 1994; Kruuk & Snell 1981). A mortality rate up to 27 % has been reported in some marine iguana populations (Kruuk & Snell 1981). Because the introduced predators have not reached all of the islands, this offers an experimental setting with a comparative approach where some marine iguana populations still live on islands without dogs or cats and others are exposed to the novel predators in recent times.

We measured flight initiation distances (FIDs) of marine iguanas as a measure of anti-predator behavior in lizards by conducting standardized approaches of an investigator towards the animals. This represents an established and commonly used technique to simulate predator approaches (Bulova 1994; Burger & Gochfeld 1990; Cooper 1997). We then compared FIDs and distinct escape tactics of individual iguanas at different sites with and without introduced predators and determined the influence of demographic factors such as age and sex on both parameters. Furthermore, we measured the

circulating concentrations of the glucocorticoid corticosterone (CORT) in the bloodstream, the main stress hormone of reptiles. We also measured body condition and body temperature as independent additional factors that can influence CORT concentrations (Romero & Wikelski 2001, Romero unpublished data).

Although marine iguanas experienced relaxed predation pressure through long evolutionary periods of time, we hypothesized that marine iguanas should differ in behavioral and physiological phenotypic plasticity according to the intensity of exposure with regard to introduced predators. Overall, FID and CORT concentrations should increase in marine iguanas at sites with novel predation threat. Furthermore, we expected individual variation in FID and CORT concentrations associated with the relative vulnerability to predation, for instance, younger and thereby smaller individuals should show larger FIDs and higher CORT concentrations than older and larger individuals to better cope with the stronger threat by predators due to its smaller body size.

4.3 METHODS

4.3.1 Study sites

We examined and compared anti-predator behavior between marine iguana populations from 2002 - 2005 at eight sites on six islands of the Galápagos archipelago with and without introduced predators, namely dogs and cats. Santa Fé (St. Fé), Caamaño (Caa), and Plaza Sur (Plaza) are uninhabited and pristine islands without dogs and cats. Only iguanas on St. Fé experience modest predation pressure by native Galápagos hawks *(Buteo galapagoensis)*, which mainly prey on hatchlings or nesting females. The island Santa Cruz is inhabited and has large numbers of introduced predators in some areas. We investigated three sites on Santa Cruz: 1) Punta Nuñez (PN), a 1 km long coastline in the south-east located 5 km from Puerto Ayora, where marine iguanas experience predation mainly by feral cats. This is highlighted by a population structure that is age-biased towards adult marine iguanas because cats mainly prey on small-bodied hatchlings and juveniles (Yacelga 1995); 2) the northern Santa Cruz island coast Las Bachas (LB), where the population density of marine iguanas is very low. Only few adult animals live at LB because marine iguanas usually prefer the current and nutrient-exposed southern coastlines (Laurie 1983). Predation by introduced dogs and cats at this site is uncertain, but presumably low as evidenced by few cat tracks in the sand (Yacelga, pers. comm.). Furthermore, the adult animals are not threatened by cats due to their large body size. Another site, 3) is at the southern Santa Cruz island coast close to the Charles Darwin Research Station in Puerto Ayora (ECCD = Estacion Cientifico Charles Darwin). Here, predation pressure by introduced predators is also not documented, but most likely absent as a consequence of eradication programs and the prohibition of dogs in this area. Another study site was on the inhabited island Isabela (Isa) in the western part of the archipelago close to Puerto Villamil, where dogs and cats are present but predation on marine iguanas has not been documented as of now. San Cristobal as the most eastern study site was chosen because

marine iguanas suffered high predation pressure by domestic dogs from the nearby town Puerto Baquerizo Moreno. With the exception of St. Fé, hawk predation on marine iguanas is unlikely or can be assumed as insignificant because hawks either are extirpated on those islands because of human colonization or usually prey on other species than marine iguanas (Bollmer et al. 2006; DeVries 1976; Gifford 1919).

In addition to anti-predator behavior assessed by comparing FIDs and determining alternative escape tactics such as hiding or running, we measured circulating CORT concentrations in the blood of marine iguanas at four sites on two islands without (St. Fé and Caa) and two islands with predation pressure (PN and SCr).

4.3.2 Measuring anti-predator behavior by flight initiation distance (FID) and escape tactics
We selected focal animals randomly that had not moved prior (>10 min) to the start of the experiment to measure anti-predator behavior. First, we recorded age class (hatchling = first year; juvenile = 2-3 years; adult >= 4 years) by estimating body size and morphological traits such as shape and color with binoculars from a distance in order not to affect the animal's behavior (Laurie 1983). We assessed sex of adults based on distinct differences in coloration, shape of head and spines and body size (Dellinger 1990). Second, we recorded the starting position and initial situation of the focal animals to control for possible effects regarding anti-predator behavior. This included group size, position within a group of more than five individuals (inside or edge of the group), alertness (score 0-4 with increasing alertness: 0 = head down, body flat; 1 = head up, body flat; 2 = head up, front legs somewhat lifted; 3 = head up, front legs straight; 4 = head up, front and back legs somewhat lifted), orientation of head (clock-wise: 11-1 o'clock position = animal turns away from approaching investigator; 2-4 and 8-10 o'clock position = animal remains collateral to approaching investigator; 5-7 o'clock position = animal faces approaching investigator). We then walked directly toward the animal at a slow pace (approx. 0.5 m/sec) without stopping to simulate an approaching predator. We marked the location of the observer at which the focal animal either moved for at least one snout vent length or when its alertness changed as indicated by changes in the posture of head and legs. Whenever the focal animal became alert (and thus changed its alertness level), we continued walking until the animal moved one snout vent length or more. We then marked the location and recorded the distance between investigator and animal where it became alert (first response distance) and the flight initiation distance (FID) where the animal initiated escape (physically moved away from investigator). For animals that immediately moved without prior change in alertness, first response distance and FID were identical. We also discriminated the type of response and distinguished between animals that ran away or hid in crevices, and between animals that ran short distances (1-3 m = "short sprint") or longer distances (more than 3 m = "long sprint") after they had initiated escape. To avoid pseudo-replication, we continued with our measurements moving in one direction through the colony. We always wore light colored clothing to keep our approaches visually standardized.

4.3.3 Measuring plasma corticosterone (CORT) concentrations and body condition

We used the standard capture and restraint stress protocol to measure CORT baseline and stress-induced CORT concentrations in marine iguanas described in details by Romero & Wikelski (2001) and Wingfield (1994). Marine iguanas of two age classes (juveniles and adults) at sites with different degrees of predation pressure were caught with a noose. We then took a blood sample of about 1 ml from the tail vein within 3 minutes after capture and preserved it in sodium heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). We avoided blood sampling directly after low tide when the marine iguanas had just fed in the intertidal zone because the circadian rhythm of CORT baseline is influenced by the tidal cycle and/or food intake in this species (Woodley et al. 2003). Body temperature was measured in the cloaca with a digital thermometer (accuracy 0.1°C) immediately after initial blood collection to test for its potential influence on CORT. We excluded animals with body temperatures lower than 30°C because low body temperatures can influence CORT concentrations in marine iguanas (Romero unpublished data). Iguanas were temporarily held in an opaque cloth bag. A second blood sample was collected after 30 minutes to quantify the CORT response to acute stress (restraint in a bag). Blood samples were stored for up to 6 hours at ~10°C in a thermos bottle on cooling gel packets and then centrifuged in the field (Baacklab Microcentrifuge, 6 x 1.5 ml, 6000 min −1) at 2000 g for 10 minutes. Plasma was removed from each sample and stored at 4°C for up to 7 days in a gas-fueled cooling box and was then frozen at -20°C at the Charles Darwin Research Station until the samples were transported on dry ice to the lab (Princeton University, NJ) for analysis. Each iguana was weighed to the nearest 20 g with a digital balance (Kern, CH15K20) and the snout vent length (SVL) was determined to calculate a body condition index ((body mass/SVL^3)*10^6[g/mm^3]) as a measure of the physical condition of the animal (Laurie 1989). Iguanas die at indices of less than ≈25 and are maximally fat at indices of 60 (Romero & Wikelski 2001). After the above procedure, we marked the animals with a synthetic paint mark that keeps about two months to avoid pseudo-replication.

With the exception of San Cristobal, we investigated the anti-predator behavior of marine iguanas exclusively during the reproductive season from October to January 2002/03 and 2003/04 to minimize possible seasonal effects. CORT concentrations were only measured in the reproductive period of 2003/04. Animals on San Cristobal were studied from February to March 2005 shortly after reproduction because acute dog attacks occurred at this time which offered an ideal option to study marine iguanas under acute predation threat.

All procedures on iguanas were conducted in accordance with the guidelines set forth by the American Society of Ichthyologists and Herpetologists and were approved by the Princeton Institutional Animal Care and Use Committees in reference to the NIH standards for the use of animals in research. We comply and work with the official permission of the Galápagos National Park Service, Republic of Ecuador.

4.3.4 Steroid assays and CORT calculations

CORT was extracted with dichloromethane and analyzed by indirect radioimmunoassay. For details on methodology see Wingfield and Farner (1975), Wingfield et al. (1992), and Romero and Wikelski (2001). We used CORT antibody, B3-163, (Esoterix Endocrinology, Austin, TX) and assayed each sample in duplicate with 20 ul of radio-labeled steroid that we added to determine recovery. Intra- and inter-assay variation of the hormone assays was on average 3.1 % and 18.76 % with a detection limit of 1.04 ng/ml.

We reported CORT baseline and response concentrations after acute stress (restraint in a bag) as well as the change from baseline to response concentrations (amplitude). We also report the "area under the curve", that is the integral of the CORT response relative to an individual's CORT baseline as the total amount of CORT released in response to a stressor (integrated response) (Dallman & Bhatnagar 2001; Sapolsky 1992). Biological effects result from interactions with specific hormone-receptors that occur over the entire course of the stress response and not just at the peak of hormone release (reviewed by Romero 2004).

4.3.5 Statistical analysis

We used General Linear Models (GLM) and Bonferroni post hoc pair wise comparison to investigate site effects on FIDs. Because the four co-variables group size, position within group, alertness and orientation did not have any significant effects on FIDs (group, $F = 1.399$, $p = 0.239$; alertness, $F = 0.469$, $p = 0.495$; orientation, $F = 3.447$, $p = 0.065$; site, $F = 3.447$, $p < 0.001$, $R^2 = 52.3$ %), we excluded these variables from further analysis. Separate GLMs were used to compare FID and CORT concentrations between animals at different sites and to assess potential effects of age and sex. We applied Repeated Measures General Linear Models (RM-GLM) for comparison of the entire stress response of animals at different sites (within-subject factor: time, between-subject factor: site), including the two samples that were taken immediately after capture and after 30 minutes of restraint.

Frequencies of several escape tactics, namely "response type" (hiding or running), "distance of sprint" ("short sprint" = 1-3 meter or "long sprint" = more than 3 meter), "first response distance" (equal to FID or larger than FID), were compared with cross-tabulation and Chi-Square test between marine iguanas assigned to three groups: one without novel predators (Santa Fé, Caamaño, Plaza Sur), another with undocumented status (ECCD, Isabela, Las Bachas) and a third with predation pressure by introduced predators (Punta Nuñez and San Cristobal). We applied Mann-Whitney U tests to compare FIDs between marine iguanas with different escape tactics. Whenever necessary, FID and CORT concentration were log-transformed to achieve normality and homogeneity of variance. Data were analyzed with SPSS for Windows 10.0 and α was set to $p = 0.05$.

4.4 RESULTS

4.4.1 FID

We present FID data only, because the first response distances were highly positively correlated with FIDs (Pearson Correlation = 0.746, p < 0.001, n = 274) and showed the same pattern as FIDs. FIDs differed significantly between sites with different degrees of predation threat ($F_{7,148}$ = 22.9, p < 0.001). Marine iguanas at sites with predation pressure by dogs and cats (Punta Nuñez and San Cristobal) showed larger FIDs compared to sites without or with undocumented predation pressure (Fig. 1).
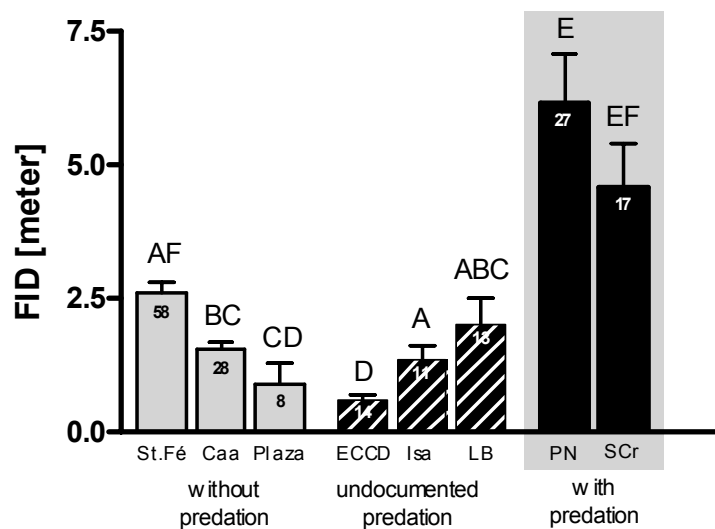
Fig. 1 Flight initiation distances (FID) of adult marine iguanas at 8 sites without, with undocumented and with predation pressure on marine iguanas by introduced predators. Different letters above bars indicate significant differences between sites (p < 0.05) based on Bonferroni post hoc pair wise comparison. Numbers within bars present sample sizes. Means ± 1SE are presented.

Tab. 1 General Linear Models (GLM) of FIDs from marine iguanas of **A)** three age classes (hatchlings, juveniles, and adults) and **B)** both sexes. Animals of 5 different sites without, undocumented and with predation pressure are included.

GLM (FID)	df	F	p
A) age class	2,476	10.492	< 0.001
site	4,574	23.778	< 0.001
age class * site	7,476	2.148	0.038
R^2 = 44.8 %			
B) sex	4,346	48.699	< 0.001
site	1,346	4.069	0.044
sex * site	3,346	5.203	0.002
R^2 = 44.5 %			

FIDs also differed significantly between sites without predation by introduced species. Marine iguanas on St. Fé, where hawk predation exists, showed larger FIDs compared to Caamaño and Plaza Sur without hawk predation (Fig. 1). Unexpectedly, FIDs were significantly shorter in marine iguanas at ECCD, a site with undocumented predation threat but regular human appearance than the two other sites, Las Bachas and Isabela, with undocumented predation (Fig. 1).
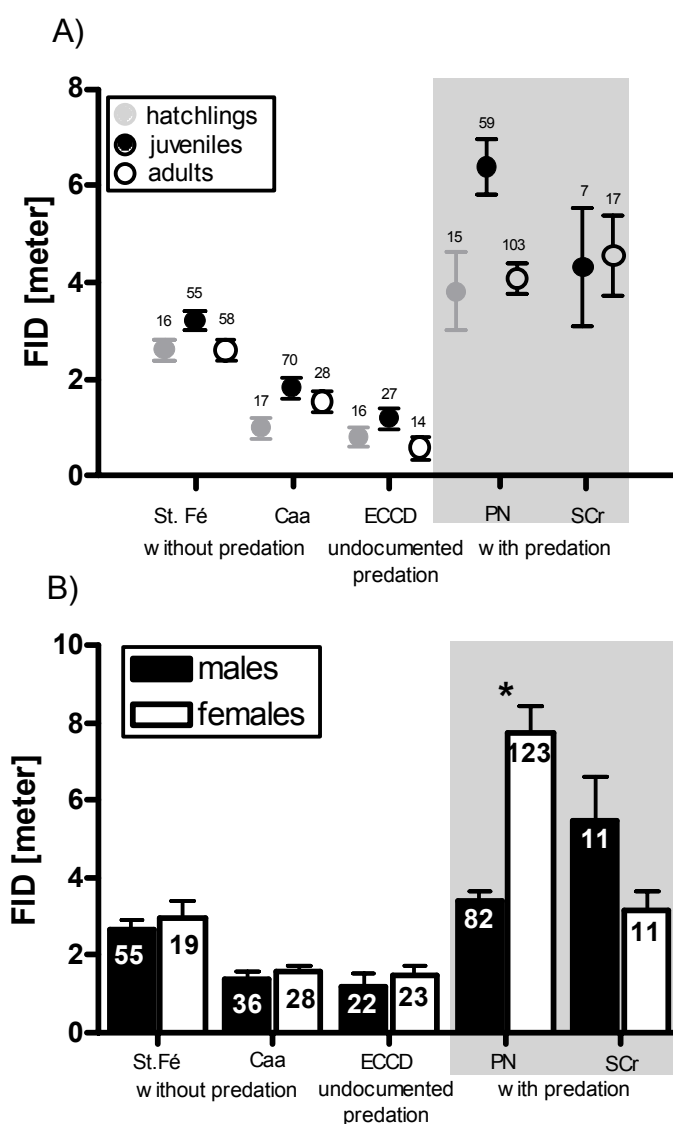
Fig. 2 Flight initiation distances (FID) of marine iguanas with different **A)** age (hatchlings, juveniles and adults) and **B)** sex, at sites with different magnitudes of predations threat (without, with undocumented and with predation). Due to acute predation by introduced species, there were no hatchlings available at San Cristobal. Numbers above and within bars, respectively, indicate sample size. Means ± 1SE are presented. * $p < 0.05$ (for statistics, see Tab. 1).

Furthermore, our data revealed differences in FID within sites that are associated with age and sex of the marine iguanas. Juveniles fled at significantly longer distances than hatchlings and adults at all sites except San Cristobal (Fig. 2 A, Tab. 1). At Punta Nuñez, we found the most pronounced FID differences between marine iguanas of different age classes (Fig. 2 A, Tab. 1). We applied two separate GLMs with different data subsets for investigating age and sex effects to avoid low power by unbalanced sample sizes of groups. Our data demonstrated that only marine iguanas at Punta Nuñez showed a significant sex difference, i.e. female marine iguanas had longer FIDs than males, in contrast to all other sites where no significant differences in sex were found (Tab. 1, Fig. 2 B).

4.4.2 Escape tactics

Overall, few individual iguanas hid into crevices when we approached them in a standardized way (Fig. 3 A, see sample sizes on bars). As predicted, the proportion of hiding animals was significantly larger (chi-square test: $\chi^2 = 12.93$, df = 2, p = 0.002) at sites with actual (7.3 % of 316) and undocumented predation (5.8 % of 121) compared to sites without predation (1.7 % of 362). A subset of marine iguanas that responded only with running, a larger proportion showed a long sprint after initiating escape (distance of sprint: Chi-square test: $\chi^2 = 21.79$, df = 2, p < 0.001) at sites with predation (17.7 % of 170) than at sites without (4.6 % of 238) or with undocumented predation threat (4.7 % of 64).

FIDs differed significantly between marine iguanas that used different escape tactics (Fig. 3) despite the low number of escape strategies such as hiding and long sprint that caused highly unbalanced samples sizes (see Fig. 3 A, B). At sites with predation, animals that preferred hiding had concomitantly larger FIDs than runners (Fig. 3 A, z = -2.364, p = 0.018). Furthermore, at all sites independent of the degree of predation threat, animals that showed a long sprint after initiating escape had also significantly larger FIDs (Fig. 3 B, site without predation: z = -2.515, p = 0.012; with predation: z = -4.091, p < 0.001; with undocumented predation: z = -2.213, p = 0.027).

Finally, we compared how many animals moved away immediately without remaining stationary and showing signs of response, such as changes in body orientation or in contact to surface (first response distance = FID) to the number of animals that allowed an approaching investigator to get closer before switching from signs of response into actual escape (first response distance > FID). We found no differences in numbers of animals escaping at sites without, with and of undocumented predation pressure by introduced predators (chi-square test: $\chi^2 = 3.084$, df = 2, p = 0.214). At sites with predation pressure as well as areas without predation, individuals that ran more often already during the approach without first signs of response had concomitantly larger FIDs than individuals that changed alertness first before switching into actual escape (site without predation: z = -6.121, p < 0.001, with predation: z = -4.274, p < 0.001, Fig. 3 C).

We also investigated details of escape tactics relative to age classes at all sites independent on the degree of predation pressure. Hatchlings showed the hiding tactic in response to an approach of an

investigator more frequently (15.3 % of 59) than juveniles (4.8 % of 187) or adults (9.1 % of 538) (chi-square test: χ^2 = 18.305, df = 2, p < 0.001). Furthermore, hatchlings ran more often without first signs of alertness than other age groups (chi-square test: χ^2 = 23.615, df = 2, p < 0.001), that is their distance at first response signs equaled FID in 66.7 % of 81 for hatchlings, 44.5 % of 346 for juveniles, and 59 % of 602 for adults.
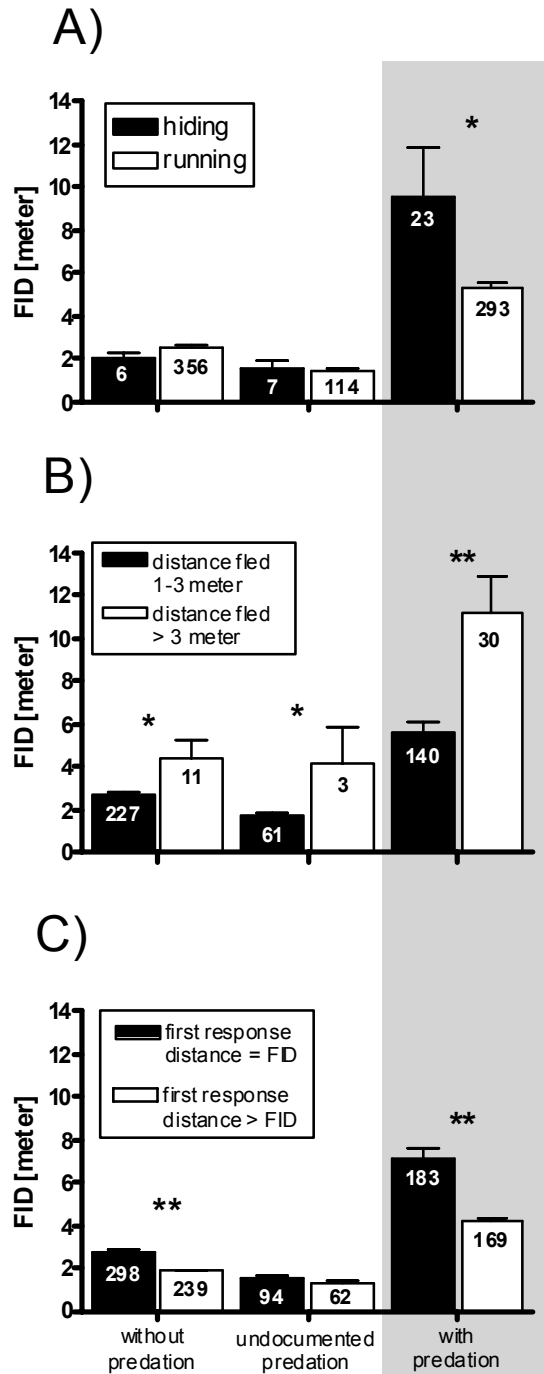
Fig. 3 Flight initiation distances (FID) of marine iguanas that use different escape tactics: **A)** hiding (black bars) or running (white bars). Panel **B)** is a subset of panel A), which only contains running animals that either fled only for a distance about 1-3 meters (black bars) or more than 3 meters (white bars) after initial flight. **C)** Individuals that either immediately initiated flight without showing alertness changes (first response distance = FID) or showed an alertness change first before initiating flight (first response distance > FID). Animals of all sites are included and sorted into three groups: without, with undocumented and with predation threat. Numbers in bars represent sample sizes. Means ± 1SE are presented (** p < 0.001, * p < 0.05).

4.4.3 CORT concentrations

All marine iguanas showed a significant increase in CORT independent of age and site with regard to a standardized stress protocol (RM-GLM: adults and juveniles: time effect, $p < 0.001$, Fig. 4, Tab. 2). Baseline CORT concentrations did not differ significantly between animals at sites with and without predation (site: $F_{3,60} = 2.434$, $p = 0.074$), but were influenced by age (age: $F_{1,60} = 12.135$, $p < 0.001$, Fig. 4). Juveniles at all sites showed higher baseline CORT concentrations than adults. However, the adrenocortical response to acute stress (CORT response concentration) differed significantly between marine iguanas at different sites (site: $F_{3,60} = 13.573$, $p < 0.001$) as well as marine iguanas of different age (age: $F_{1,60} = 35.586$, $p < 0.001$, Fig. 4). Concentrations of CORT responses were highest in marine iguanas at sites with introduced predation.

Tab. 2 Repeated Measures General Linear Models (RM-GLM) of CORT concentrations (CORT baseline and CORT response) from adult and juvenile marine iguanas at 4 sites without and with predation pressure.

RM-GLM (CORT adults)	df	F	p
Time	1,43	92.908	< 0.001
Site	1,43	6.374	0.001
time * site	3,43	11.832	< 0.001
RM-GLM (CORT juveniles)			
Time	1,15	101.15	< 0.001
Site	2,15	11.321	0.001
time * site	1,15	2.37	0.128

Tab. 3 General Linear Model (GLM) of the total amount of circulating CORT after response to acute stress (area under the curve) from marine iguanas of three age classes (hatchlings, juveniles and adults) at sites without and with predation pressure.

GLM (CORT, area under the curve)	df	F	p
age class	2,45	6.908	0.002
Site	1,45	32.496	< 0.001
age class * site	2,45	0.289	0.75
R^2 = 46.5 %			

Furthermore, they were also higher in juveniles than adults but independent of site. Hence, the total amount of circulating CORT excreted during the response to acute stress (area under the curve) was strongly influenced by age and site (site, $p = 0.002$; age, $p < 0.001$, site × age, $p = 0.75$, Fig. 5, Tab. 3). Its pattern resembled the CORT response concentrations (Fig. 5). Furthermore, the changes from base-

line CORT to acute stress levels (amplitude) of adult and juvenile marine iguanas differed significantly between sites without and with predation pressure (RM-GLM, interaction effects: adults, time × site, $p < 0.001$; juveniles, time × site, $p = 0.001$, Fig. 4, Tab. 2). However, there were no differences in CORT baseline concentrations or the adrenocortical responsiveness to acute stress in relation to sex.
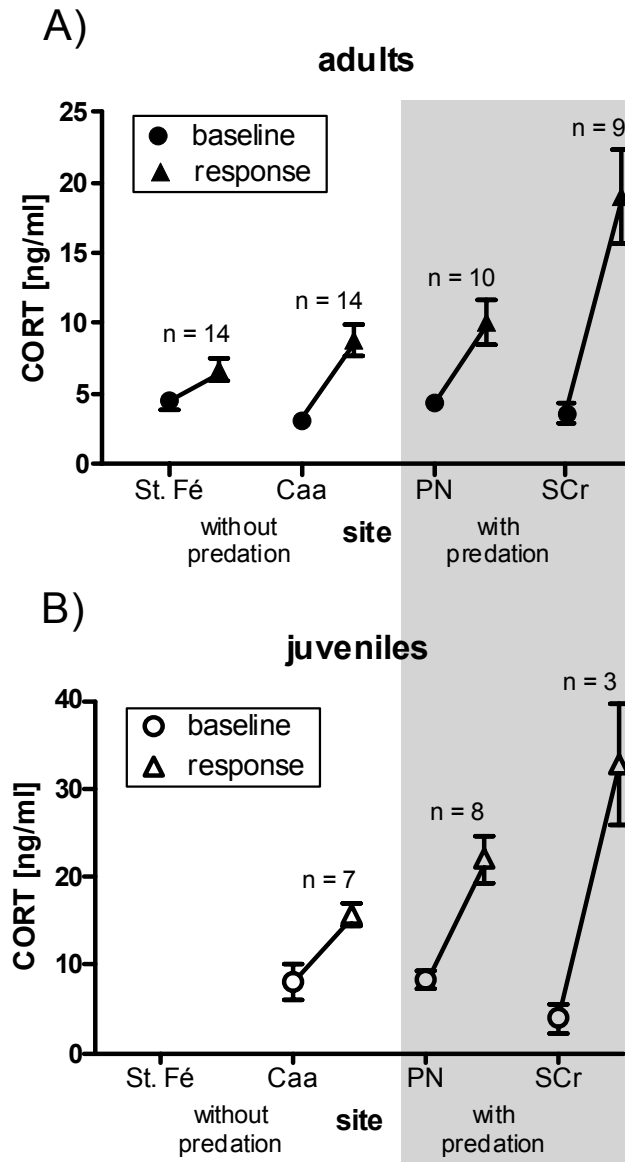
Fig. 4 Baseline CORT and CORT response concentrations of marine iguanas of two age classes, **A)** adults and **B)** juveniles, at sites without and with predation threat. Means ± 1SE are presented (for statistics, see Tab. 2).

Body condition of marine iguanas differed significantly between sites, but there was no age effect on body condition (site, df = 3,61, F = 8.478, $p < 0.001$; age, df = 1,61, F = 1.686, $p = 0.199$; site × age, df = 2,61, F = 1.042, $p = 0.359$). At sites with predation pressure, marine iguanas had the highest body

condition indices (Punta Nuñez: 56.8 ± 7.2 adults, 52.5 ± 3.8 juveniles; San Cristobal: 54.5 ± 6.5 adults; 51.7 ± 4.7, juveniles; St. Fé: 47.3 ± 3.9 adults, data for juveniles not available; Caamaño: 48.2 ± 5.2 adults, 48.6 ± 5.7 juveniles). Thus, the elevated adrenocortical responsiveness at sites with introduced predators was not associated with low body condition in our study animals.
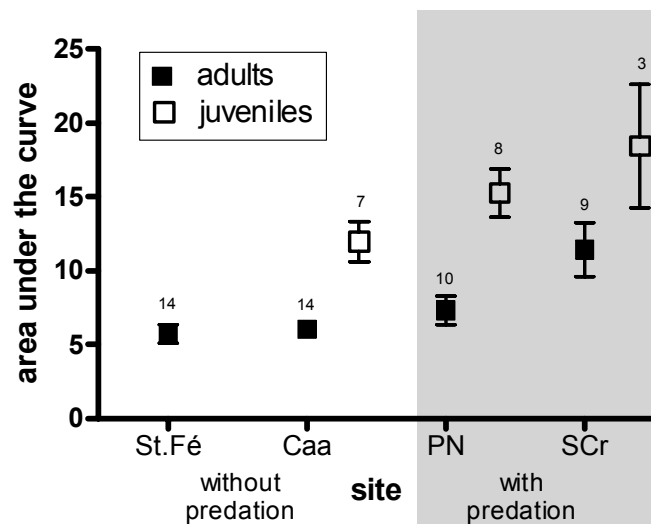
Fig. 5 The total amount of circulating CORT after response to acute stress (area under the curve) in adult and juvenile marine iguanas at sites without and with predation threat. Numbers above bars represent sample sizes. Means ± 1SE are presented (for statistics, see Tab. 3).

4.5 DISCUSSION

4.5.1 Anti-predator behavior – the influence of predation

Our results provide evidence that marine iguanas as a "naïve" island species are at least partially capable to resurrect or develop anti-predator behavior as a consequence of a new predation threat, even if they have been isolated from large predators for several millions of years. In our study, the presence of introduced predators (dogs, cats) was associated with increased FIDs and qualitative changes in alertness of marine iguanas such as hiding in crevices and long sprints. This suggests that about 150 years of experience with predators were sufficient to modify anti-predator behavior in response to a novel threat.

It is not known whether these adjustments have a genetic component or are mainly caused by individual learning experience. We hypothesize that behavioral learning contributed most to the observed plasticity based upon the long generation time of marine iguanas of up to 30 years (Berry 1984) combined with the short time-frame of a maximum of 200 years since the introduction of dogs and cats. The behavioral learning hypothesis is also consistent with the high variability of FIDs in marine

iguanas at sites with introduced predators (from 0 up to 54.5 meters). Adjustments of anti-predator behavior by learning or through natural selection are not mutually exclusive, thus no final conclusion can be drawn at this point. Nevertheless, there was a strong suggestion for rapid adaptive responses to environmental changes in a recent study where Australian black snakes *(Pseudechis porphyriacus)* adapted their feeding behavior and physiological resistance to an introduced prey species, the toxic cane toad *(Bufo marinus)*, in fewer than 23 generations (Phillips and Shine 2006). Additional laboratory studies suggested that those changes were not attributable to learning but have evolved in response to the presence of toads (Phillips & Shine 2006).

Although all marine iguanas reacted to the new predator threat to a certain degree, the strength of the reaction was very low in our study animals. Only 7.3 % of the individuals studied hid and 17.7 % showed a long sprint during a simulated predator approach. Furthermore, the iguanas' average FIDs of only 5.4 meters at sites with introduced predators were minor compared to FIDs of some mainland iguanas, e.g. the spiny-tailed iguanas *(Ctenosaura hemilopha)* that already ran away at an average distance of 30.9 meters (Blazquez et al. 1997). To conclude, although the marine iguanas adjust anti-predator behavior to increased predation pressure, they are unlikely to significantly lower the risk of predation by dogs and cats because the adjustments are minor. This conclusion is supported by the high mortality of marine iguanas on San Cristobal, where we found over 150 dead corpses along a 2 km coastline, apparently killed by infective bites through domestic stray dogs from a nearby town.

Furthermore, marine iguanas seemed to show a slight adjustment in FIDs to a native predator, the Galápagos hawk *(Buteo galapagoensis)*, at least on Santa Fé where a few hawks occasionally prey on marine iguanas. Iguanas from Santa Fé fled at significantly larger distances during investigator approaches than on the hawk-free islands of Plaza Sur and Caamaño, which are also free of introduced predators. Genetic analyses indicate that the hawks colonized Galápagos rather recently, likely less than 300,000 years ago (Bollmer et al. 2006). In contrast, marine iguanas have probably diverged from their sister taxa, the land iguana *Conolophus sp.*, 10 – 20 million years ago on Galápagos islands, which sank in the meantime (Rassmann 1997). Although 300,000 years of co-existence between hawks and marine iguanas could be enough for behavioral adjustments on a genetic basis, the question remains whether the predation pressure on marine iguanas is suffice to develop the capability to perceive hawks as a serious threat. Only 14-16 hawk territories occur on Santa Fé and only about 3-4 of them include marine iguana colonies (DeLay et al. 1996). Furthermore, hawks often prey on other species than marine iguanas (DeVries 1976). However, support for behavioral adjustments on a genetic basis is provided by observations of marine iguana hatchlings that probably never had negative experiences with hawks before, but sprinted away and tried to hide in crevices during hawk approach. In contrast, adult marine iguanas hardly responded when a hawk walked through their colony (Berger and Rödl, pers. observation).

Marine iguanas exhibited signs of habituation to human exposure at the Charles Darwin Station on Santa Cruz, a site where they are often subject to repeated human disturbance by tourists,

locals and fishermen. Marine iguanas there showed the smallest FIDs from all sites. Habituation to human disturbance in iguanas was also shown by Burger and Gochfeld (1990) who found that black iguanas *(Ctenosaura similes)* living in areas of intense human exposure allow closer approach than those living in areas of infrequent or no human interference. Probably animals, which experience repeated adverse stimuli in nature would risk wasting energy in terms of costs for running or loosing opportunities for foraging and reproduction (Ydenberg & Dill 1986) if they responded unnecessarily. Thus, selection is likely to favor abilities for accurately assessing the threat of potential predators (Burger & Gochfeld 1990). The fact that marine iguanas allow investigators to approach them more closely at sites with frequent human exposure may suggest an ability to distinguish between potential threats and harmless encounters.

4.5.2 Anti-predator behavior and individual characteristics

The individual differences of FIDs in marine iguanas with regard to their age suggest that anti-predator behavior is associated with their relative vulnerability to predation. Juveniles at all sites except San Cristobal showed the largest FIDs compared to hatchlings and adults. We hypothesize that juveniles develop anti-predator behavior because they are smaller and thus more vulnerable to native and introduced predators. Juveniles probably learn by experience to recognize potential threats and to respond accordingly, provided that they are not all killed by the attack of a predator. In such cases, a genetic basis can not be excluded. In contrast, hatchlings showed similar small FIDs than adults, possibly because they lack the experience with predators.

Hatchlings exhibited some specific behavioral reactions after approach of an investigator as they hid more often in crevices than adults. This might be a compensation for their increased vulnerability to predation and their size-related speed limitations. However, we observed hiding hatchlings only rarely and thus assumed a high predation pressure on the hatchlings, especially at sites with introduced predators. Indeed, this is reflected in the highly skewed age structure of local populations where young cohorts are largely missing in areas with introduced predators (Kruuk & Snell 1981; Laurie 1983). On San Cristobal and Santa Cruz, populations included less than 1 % of hatchlings and juveniles, compared with up to 30 % in colonies on Santa Fé (Laurie 1983).

Furthermore, we also found sex-specific differences in FID at Punta Nuñez with introduced predators where females showed larger FIDs than males. Because the smaller females (SVL = 320 ± 24.1 mm) may be more threatened by dogs and cats than the larger males (SVL = 414 ± 40.4 mm), sex differences in anti-predator behavior could be a consequence of sex differences in body size (Shine et al. 2000). In contrast, female marine iguanas on San Cristobal did not react towards the approach of an investigator at larger distance than males. However, sample sizes of this site could be too small to detect significant sex differences in FID.

Alternatively, individuals of distinct sexes may be under different selection pressures which can cause differences in FID. Hence, sexual selection may favor males with short FIDs to minimize

time away from the territory due to unnecessary escape (Snell et al. 1988). Territorial males that escape for instance to avoid potential predators face an obvious conflict because a fleeing animal cannot defend its territory from conspecific intruders and thereby might loose its territory and thus chances for successful copulations (Díaz-Uriarte 2001). Therefore, males exhibit shorter FIDs when costs of lost reproductive opportunities, such as mating or fighting sexual competitors, are greater (Cooper Jr. 1999) according to the optimal escape theory (Ydenberg & Dill 1986). Such a trade-off between reproductive behavior and anti-predator behavior has been observed in mainland species (Cooper Jr. 1999; Diaz-Uriarte 1999). However, the question remains whether 200 years of co-existence between island species and introduced predators would be enough to develop such a trade-off.

4.5.3 CORT concentrations and the influence of predation

On San Cristobal and Punta Nuñez, where marine iguanas experienced predation pressure by dogs and cats, CORT response concentrations and the total amount of circulating CORT after acute stress (area under the curve) were significantly higher compared to those from animals living without introduced predators. Furthermore, the changes from baseline CORT to response concentrations after acute stress (amplitude) were most pronounced in marine iguanas at San Cristobal, the site with acute predation pressure. Therefore, our results suggest that introduced predators induced a sensitization in the adrenocortical responsiveness to acute stress (here restraint in a bag) of marine iguanas. Such a sensitization may indicate that other stressors could also be perceived in a more negative way than from an animal with a lower responsiveness. Higher sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis to stressful events is known to be modulated based upon previous exposure to stressful stimuli like abiotic and biotic stressors (Smith et al. 1994). This modulation might present a mechanism for adjusting the physiology to cope with the impact of stressors. CORT is capable of inducing transient behaviors, particularly activity that promotes escape and ultimately survival by getting the animal away from a stressor (Breuner et al. 1998). In general, the rapid actions of CORT on metabolism may have obvious fitness consequences (Orchinik 1998), a useful adaptation to dangerous and unfavorable environments.

Hormone profiles are important tools in conservation to determine the impact of different types of human disturbance on the physiology of wild animals (Müllner et al. 2004; Wikelski & Cooke 2006; Wikelski et al. 2001; Wingfield et al. 1995). As an example, Magellanic penguin *(Spheniscus magellanicus)* and hoatzin chicks *(Opisthocomus hoazin)* had higher CORT response concentrations in areas visited by tourists than in undisturbed areas (Müllner et al. 2004; Walker et al. 2005). Another example showed that baseline CORT and CORT response concentrations were significantly higher in marine iguanas after an oil spill than in animals measured before the spill (Wikelski et al. 2001). The authors suggested that CORT was elevated because iguanas ingested oil residues while feeding in polluted intertidal areas.

Interestingly, in our study, baseline CORT concentrations that are often used as an indicator of chronic stress (Wingfield et al. 1998) were similar for all marine iguanas at different sites. However, CORT response concentrations to acute stress (restraint in a bag) were elevated in individuals at sites with introduced predators. This may indicate that predation did not lead to chronically elevated levels of CORT (Romero et al. 2004), but caused a higher adrenocortical responsiveness towards certain stimuli in those animals, which were exposed to potential predators as compared to others, without.

4.5.4 CORT concentrations and individual characteristics

Both, the total amount of circulating CORT after acute stress (area under the curve) and the CORT baseline and response concentrations were significantly higher in juvenile marine iguanas than in adults. This pattern was very consistent across sites and suggests that age contributes to adrenocortical variation (Dunlap 1995). A similar pattern has been reported for juvenile green turtles *(Chelonia mydas)*, which had higher basal plasma levels of CORT and showed a stronger increase in CORT to acute stress than adults (Jessop & Hamann 2005). The authors proposed that the higher adrenocortical responsiveness might be adaptive for juveniles to counteract the lower survivorship due to a higher predation risk.

Higher glucocorticoid levels can support animals to cope better with predation. Increased vigilance and rapid responses to potential threats by glucocorticoids enhance chances of survival (Boinski et al. 1999) as they help to escape from predators. This fits into the concept that elevated glucocorticoid concentrations are crucial in regulating behavior and physiology of animals in particular life-history stages (Guillete et al. 1995; Wingfield et al. 1998). Juvenile marine iguanas face a higher predation risk by native and/or introduced predators because of their small body size. Consequently, increased basal CORT concentrations may prepare the juveniles to cope better with the elevated predation threat (Sapolsky et al. 2000) as they can react faster with an escape response.

However, in reptiles, higher basal levels of CORT are often associated with increased energetic requirements for somatic or reproductive processes (Moore & Jessop 2003). Thus, endocrine differences in marine iguanas also may reflect different activity levels of juveniles compared with older and larger size classes independent on the vulnerability to predation.

4.5.5 Conclusions

Our results suggest that introduced predators on Galápagos are possibly responsible for an elevation in wariness and adrenocortical responsiveness of marine iguanas. We propose that marine iguanas are capable to resurrect or quickly develop anti-predator responses within less than 200 years in spite of an absence of predators throughout several million years. As the high mortality of marine iguanas at sites with novel threats (cats, dogs) indicates, the observed increase in wariness of the marine iguanas was not sufficient to prevent predation (Barnett 1982; Cayot et al. 1994; Kruuk & Snell 1981).

High mortality rates have also been documented for other iguanas on islands where dogs and cats had been introduced such as *Cyclura carinata* on Caicos Islands and *Brachylophus vitiensis* on Fiji. The iguanas' development of more cautious behavior was obviously not successful to prevent heavy predation caused by the introduction of novel predators (Gibbons 1984; Iverson 1978). Overall, the naivety of the respective animals and the shortcomings in their behavioral adjustments, either by slow learning or the long time until genetic fixation, make insular reptiles very vulnerable to exotic predators that have already decimated island populations of reptiles worldwide (Atkinson 1989). For example, an adult iguana population of about 5,500 on Pine Cay (Caicos islands) was nearly extirpated by dogs (Iverson 1978).

Ultimately, the question remains whether the behavioral and adrenocortical adjustments observed in the marine iguanas represent evolutionary changes due to natural selection, where only animals with higher responsiveness and/or adjustments made in response to predation have a higher chance of survival,. Alternatively, the variations in behavior and CORT levels could be part of the phenotypic plasticity of the species.

For conservation programs, the range of reaction norms in the anti-predator responses of naïve animal species on predator-free islands is essential to predict how they deal with novel threats. Furthermore, this knowledge can provide a crucial base for re-introduction programs to train animals to cope with exotic predators (Maloney & McLean 1995). However, plasticity in anti-predator responses is depending on several factors such as time period of co-existence with the predator, strength of predation threat, individual experience and local situation. This suggests that future scenarios and consequences in case of new introduced predators on islands are very difficult to predict.

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Fig. 1 Location of study sites in the Galápagos archipelago for the investigation of the influence of introduced predators on behavioral and physiological stress responses in marine iguanas. Santa Fé, Plaza Sur, and Caamaño represented sites without introduced predators. On Isabela, ECCD and Las Bachas predation was undocumented. There was heavy/acute predation by introduced dogs and cats on San Cristobal and Punta Nuñez.

Fig. 2 A, B On four inhabited islands of the Galápagos archipelago, introduced domestic dogs and cats constitute novel predators since about 100 to 150 years and have become a serious threat to native animals. Some have become independent feral populations, which hunt marine iguanas. Furthermore, there are stray dogs and cats close to human settlements, which venture into nature, uncontrolled by their owners.

Fig. 3 In March 2005 numerous marine iguana corpses were found next to the town Puerto Baquerizo Moreno on the island San Cristobal. Closer investigation and autopsy of two newly dead adult males by veterinarians revealed bite marks by dogs and secondary infections as the most likely cause of death.

Fig. 4 Several living adult marine iguanas had bite marks in their tail. These wounds were not lethal per se but resulted in serious secondary infections and, as in this case, in necrosis of large parts of the tail, finally causing death to the animal. Stray dogs from town, which do not depend on marine iguanas for food, caused secondarily death to the animals through invasive playful behavior.

Fig. 5 A) Two adult marine iguanas during 30 minutes of restraint stress in a cloth bag as described in the standard capture and restraint stress protocol by Wingfield (1994). **B)** A blood sample of about 1 ml from the tail vain into a sodium heparinized Vacutainer tube was taken within 3 minutes after capture and after 30 minutes of restraint stress.

Fig. 6 A) Determination of the snout vent length (SVL) and **B)** weight to calculate the body condition index (body mass/SVL^3)*10^6[g/mm^3]. **C)** Marking with a synthetic paint mark that keeps about two months.

Chapter 5

Tameness and stress physiology in a predator-naïve island species confronted with novel predation threat

Thomas Rödl, Silke Berger, L. Michael Romero, and Martin Wikelski

5.1 ABSTRACT

Tame behaviour, i.e. low wariness, in terrestrial island species is attributed to low predation pressure. However, we know nothing of its physiological control and little of its flexibility in the face of predator introductions. Marine iguanas on the Galápagos Islands are an excellent model to study the physiological correlates of low wariness. They have lived virtually without predation for 5 to 15 million years until some populations were confronted with feral cats and dogs beginning some 150 years ago. We tested whether and to what extent marine iguanas can adjust their behaviour and endocrine stress response to novel predation threats. Here we show that a corticosterone stress response to experimental chasing is absent in naïve animals, but is quickly restored with experience. Initially low wariness also increases with experience, but remains an order of magnitude too low to allow escape from introduced predators. Our data suggest that the ability of marine iguanas to cope with predator introductions is limited by narrow reaction norms for behavioural wariness rather than by the underlying physiological stress system. We predict that island endemics generally may show flexible physiological stress responses but are restricted by narrow behavioural plasticity.

5.2 INTRODUCTION

In the absence of predators, island habitats allow for physiological and anatomical adaptations, e.g. flightlessness (McNab 1994), which reduce the capability to escape once predators are introduced. In contrast to such hard-wired traits, behavioural patterns, e.g. the prominent tame behaviour in island endemics (Blumstein 2002), should be more flexible such that experience-dependent anti-predator behaviour can be quickly regained (Berger et al. 2001). This flexibility is the focus of many re-introduction programs training naïve animals to recognize and cope with introduced predators (Beck et al. 1994; Maloney & MacLean 1995; Griffin et al. 2000). Still, in most cases it is difficult to predict the behavioural plasticity of insular animals that are in contact with introduced predators, because our understanding of the physiological mechanisms controlling anti-predator behaviour such as flight is highly deficient. Flight is usually accompanied by a physiological stress response, which is thought to promote the success of flight (Hofer & East 1998). A major endocrine regulatory unit of the stress response, the hypothalamo-pituitary-adrenal (HPA) axis, is represented by corticosteroid hormones in the blood plasma, which can be sampled under field conditions (Sapolsky et al. 2000). In species accustomed to the presence of local predators, detection of a predation threat is known to activate the HPA axis and to increase circulating plasma corticosteroid concentrations (Scheuerlein et al. 2001; Cockrem & Silverin 2002) after a latency of about three minutes (Romero & Reed 2005). Nothing, however, is known about the interactions between wariness and the endocrine stress response in free-ranging tame animals (those not accustomed to the presence of predators).

Here we investigate the physiological background of tame behaviour, i.e. low wariness. We experimentally test the behavioural and physiological responses of an island endemic to a novel threat using local contrasts in predation risk and an unusually high incident of predation – a setting that allows replication of our experiments at three sites with differing predation pressure by introduced mammals. We chose the Galápagos marine iguana *(Amblyrhynchus cristatus)* as a model species because of its exceptional low wariness and a large body of available background information on both the behaviour and physiology of the species.

5.3 MATERIALS AND METHODS

5.3.1 Study sites and animal selection
The small island Caamaño (S0º45'31'' W90º16'43''), 4 km off the town Puerto Ayora / St. Cruz, never had any introduced predators (Trillmich & Trillmich 1986). The population at Punta Nuñez (S0º44'45'' W90º15'17'') on the southeast coast of St. Cruz is age-biased towards adult animals due to predation (Yacelgas 1995). Both sites were chosen because of contrasting predation threats in spite of their proximity to each other (similar climatic conditions). Our study site on San Cristobal Island

(S0º55'05' W89º37'40'') is about 2.5 km west of the town of Puerto Baquerizo Moreno and 70 km from the other two sites. At the time of the study it was the site with the highest known predation pressure, suffering from acute predation by dogs possibly from the nearby town (see electronic supplementary material).

Data within each graph panel are from one season (December 2003 to January 2004 on Caamaño, March 2005 on San Cristobal), with the exception of Punta Nuñez, from where corticosterone (CORT, the principal corticoid in reptiles) values stem from November to December 2003 and March 2005, which were, however, indistinguishable (unpaired t test with naïve controls, t = 0.41, df = 24, p = 0.690). Because our site on San Cristobal is situated in a military zone, no data for the "previously caught and chased" group could be collected due to logistic constraints.

We excluded females, bachelor males and young animals to reduce variability due to sex- and status-specific characteristics (Berger et al. in prep.). However, for verification we replicated the comparisons of CORT concentrations between naïve controls and chased groups with animals between 2 and 4 years of age, which could be more prone to predation.

5.3.2 Flight initiation distances and experimental chasing

From far enough not to interfere with behaviour, a territorial male iguana was chosen randomly. One observer approached the animal in a standardised way along a straight line, with a constant speed of about 0.5 m/s, avoiding jerky movements and not wearing sunglasses as this has been shown to potentially influence responses (Burger & Gochfeld 1990). The location of the observer at which the animal started to move was marked and the shortest distance to the animal's original location defines the flight initiation distance FID (Cooper 2003). Experimental chasing is essentially the continuation of flight excitation for a period of 15 minutes. The observer halted each time the focal animal moved, to pursue approach after the animal came to a stop. Caught animals had been painted with a number for later recognition. Paint usually wears off after 6-8 weeks and is not known to harm the animals.

5.3.3 Blood sampling and processing

Adult males were sampled randomly. Up to 0.5 ml of blood was drawn with subcutaneous vacutainer needles from the caudal vein usually within 2 minutes (no longer than 3 minutes) after the first capture attempt (Sapolsky et al. 2000). Blood was stored in an insulated container with cool gels for no more than a few hours and then centrifuged at 2000 rpm for 5 minutes. The plasma was then transferred into a gas powered camping refrigerator in the field, later frozen and transported on dry ice. Laboratory analysis involved a dichloromethane extraction for steroids and a competitive binding radio immuno assay as has been described elsewhere (Wingfield et al. 1994). Average sample volume of the RIA was 50 ul, average intra- and inter-assay variation was 3.0 % and 17.23 %, respectively, and the detection limit averaged at 0.55 ng/ml.

5.3.4 Testing potential confounds

Many studies on FIDs in wild animals assume that the animals' responses towards humans reflect similar responses towards natural predators (Stone et al. 1994; Sapolsky et al. 2000; Frid & Dill 2002). We tested this assumption for both parameters used in this study, FID and CORT response to chasing in marine iguanas by comparing results from (i) a person accompanied by a trained dog on a leash approaching and chasing, respectively, with (ii) a person approaching and chasing without a dog. FIDs of animals close to Puerto Ayora, St. Cruz Island, which were approached with a dog (mean ± 1SE = 2.52 ± 1.52, n = 3) were not significantly different from animals approached without a dog (mean ± 1SE = 2.04 ± 1.41, n = 30, t = 1.329, df = 41, p = 0.191). Similarly, CORT concentrations in animals at Punta Nuñez were not significantly higher when chased by the experimenter with (mean ± 1SE = 4.81 ± 1.71, n = 10) than without a dog (mean ± 1SE = 7.32 ± 2.48, n = 8, t = 0.666, df = 16, p = 0.515).

5.3.5 Statistical analyses

The increase in CORT concentration after 15 minutes of restraint in animals from Caamaño (Fig. 1) was tested with a paired t test, all other data from within a site (Fig. 2) with unpaired t tests, vertical comparisons of the restraint group with a one-way repeated measure ANOVA, and vertical comparisons of FIDs with a one-way ANOVA and Tukey posthoc comparisons. Statistical adjustments for multiple testing (Wright 1992) were taken into account for FIDs but not for the comparisons of CORT data, because the only group repeatedly used, the naïve control, serves as CORT baseline reference. Due to heteroskedasticity all CORT data had been log-transformed before testing and wariness data additionally square-root-transformed, with the normality assumptions met as a result (Kolmogorov Smirnov one-sample test). All tests were two-tailed, alpha was set to 0.05. From the previously caught and chased group on Caamaño one obvious outlier had been removed (94.55 ng/ml) that was 13 times the standard deviation above the mean. This anomaly could be due to a sick animal, problems with the plasma, or, alternatively, it could represent a small fraction of hyper-responding individuals (according to the concept of responders and non-responders, see Blanchard 1995). Inclusion of this data point would not change the statistical interpretation of the graph.

5.4 RESULTS

As in continental vertebrates, a serious threat such as capture and subsequent restraint of marine iguanas in an opaque bag causes a significant increase of CORT. After 15 minutes, CORT concentrations are significantly elevated from pre-stressed, or baseline, levels (Fig. 1; t = 7.710, df = 12, p < 0.001). CORT responses of similar strength in marine iguanas have previously been shown to result from metabolic disruptions during an El Niño episode or an oil spill (Romero & Wikelski 2001; Wikelski et

al. 2001). Recent data also show that the magnitude of the CORT response to restraint as well as the animals' wariness (measured as the flight initiation distance or FID) differs between marine iguana populations exposed to different local predation risk (Berger et al. in prep.). Therefore, adaptation to new predators seems possible, at least to a certain degree.

To test the animals' ability to detect and respond to a novel threat, we simulated predation attempts directed at marine iguanas from three islands with animals (i) living without predators (ii) living with intermediate predation risk and (iii) living with acute, heavy predation risk from introduced dogs.
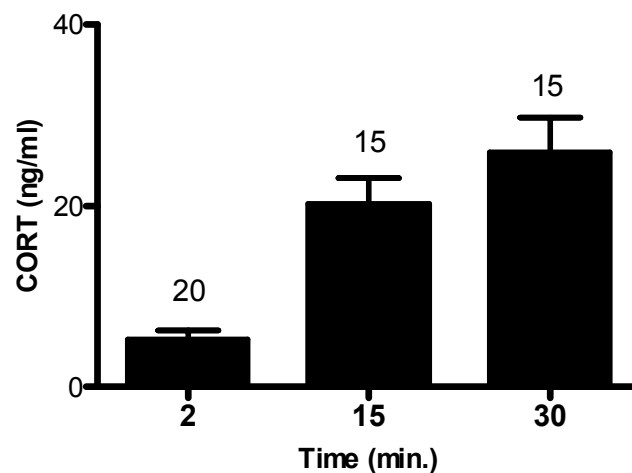
Fig. 1 Corticosterone increase after capture during restraint in an opaque bag. Corticosterone concentrations 2 minutes after capture (mean ± 1SE = 5.29 ± 0.98 ng/ml) reflect undisturbed baseline levels. 15 minutes after capture concentrations significantly increased to 20.22 ± 2.91 ng/ml.

(i) no predation

In a controlled field experiment on Caamaño Island, where marine iguanas have never experienced predation from terrestrial vertebrates, we tested the hypothesis that predator-naïve individuals not only show low wariness but congruently fail to activate the HPA-axis in response to a potential threat. In this case it was a human experimenter pursuing the focal animal for 15 minutes, without physical contact. We call this "chasing". Circulating plasma CORT concentrations in animals chased for the first time ("naïve chased") were indistinguishable from baselines in unchased "naive controls" ($t = -0.639$, $df = 13$, $p = 0.534$, Fig. 2, top right). We then tested for a learned response to new predators with another group of animals that had been caught and handled two to five weeks prior to the experiment. We subjected these individuals to a 15 min chase ("previously caught and chased"). Similar to the naïve animals, there was no significant CORT increase after chasing ($t = -1.004$, $df = 13$, $p = 0.334$). Therefore, unlike continental species, marine iguanas living without introduced mammalian

predators do not mount a CORT response to being chased; even if they had experienced capture once before. Only naïve control animals, when put in a bag after capture without prior chasing ("naïve restraint") - significantly elevated CORT concentrations, as was the case at all three sites ($F_{1,23}$ = 42.575, $p < 0.001$). Restraint is regarded as a very strong stimulus and a standard technique to test the strength of a CORT response (Wingfield & Romero 1998).

In contrast to the CORT results, FIDs on Caamaño were significantly longer in the previously caught and chased group as compared to chased animals (t = -2.935, df = 14, p = 0.011, Fig. 2, top left). A one-time prior experience was enough to increase FID in subsequent tests. In absolute terms, however, when comparing them to continental reptiles, FIDs were very short in both groups (Bulova 1994; Blazquez et al. 1997).
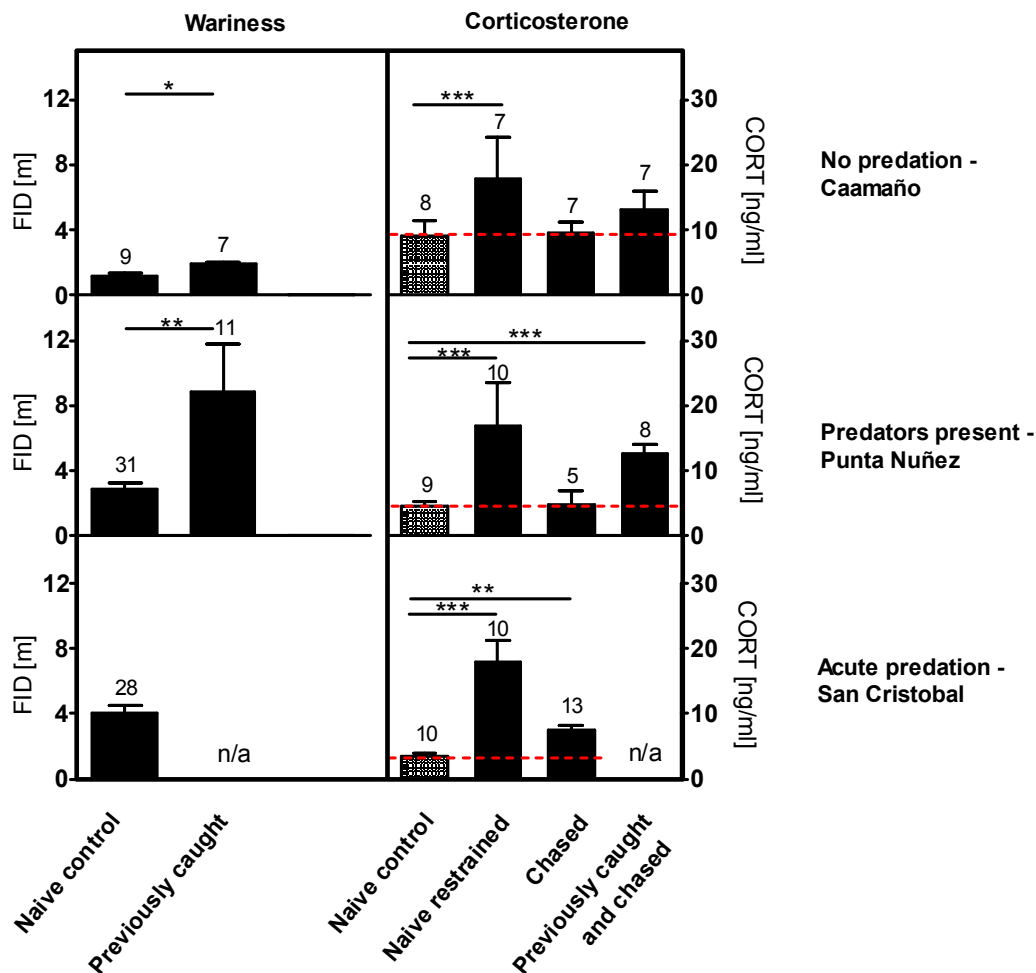
Fig. 2 Wariness and corticosterone concentrations at three sites differing in intensity of predation risk. Blood samples from "naïve control" animals were collected on average 2' after capture (i.e. CORT baselines), from "naïve restraint" animals 30' after capture (i.e. CORT response to restraint in a bag). "Chased" and "previously caught and chased" animals were subjected to 15 minutes of experimental harassment prior to capture. In contrast to naïve individuals, the latter group had been caught and handled once, two to four weeks before. Dashed lines mark CORT baseline levels (average of the "naïve control" group). All values in means ± 1SE.

(ii) intermediate predation risk

We repeated the experiment with marine iguanas that lived among introduced predators for several generations at Punta Nuñez, Santa Cruz (figure 2 middle panels). Predation at Punta Nuñez was strong in 1995 (Yacelgas 1995) but its current extent remains uncertain due to efforts to reduce cat and dog numbers by the National Park Service. As on Caamaño, CORT in chased animals was not significantly different from naive controls (t = 0.230, df = 12, p = 0.822) and far below CORT concentrations in captured and restraint animals ("naïve restraint"). However, the previously caught and chased animals at this site mounted a significant CORT response to chasing (t = -4.819, df = 15, p < 0.001). Further-more, iguanas at Punta Nuñez also had significantly longer FIDs than iguanas on predator-free Caamaño (site differences in FIDs, $F_{2,81}$ = 18.501, p < 0.001 with Caamaño vs. Punta Nuñez p < 0.001), especially in previously caught individuals (naïve controls vs. previously caught at Punta Nu-ñez, t = -3.271, df = 40, p = 0.002). Living around predators at Punta Nuñez must have primed both the behavioural and endocrine responses.
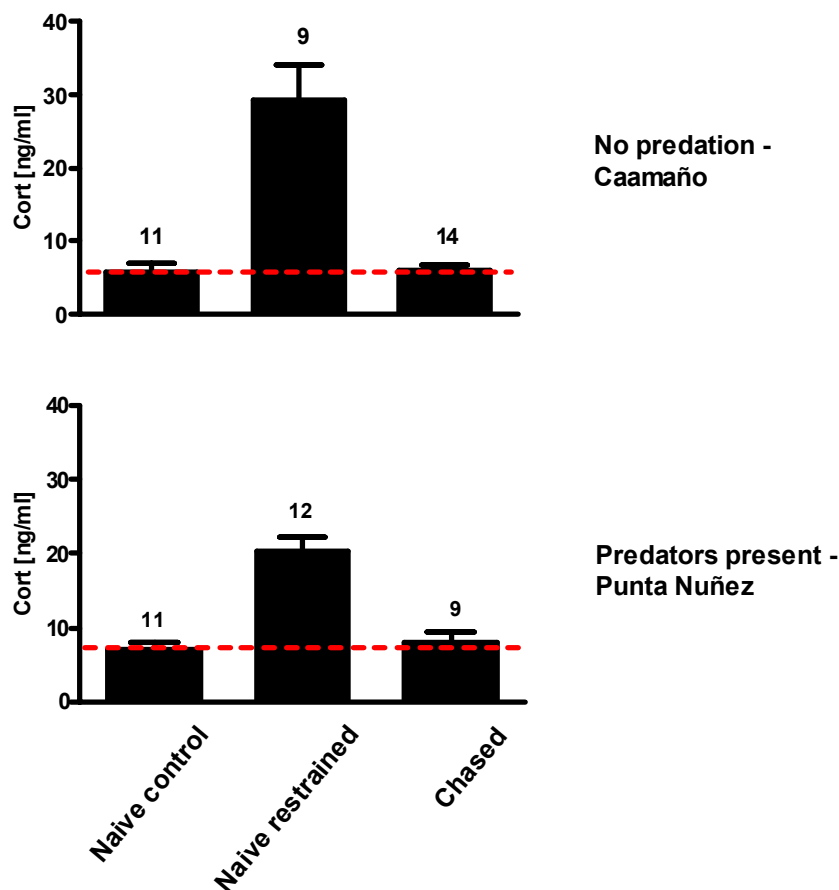
Fig. 3 CORT concentrations of *young animals* (2-3 years of age) at two sites differing in intensity of predation risk. Blood samples from "naïve control" animals were collected on average 2' after capture (i.e. CORT baselines), from "naïve restraint" animals 30' after capture (i.e. CORT response to restraint in a bag). "Chased" animals were subjected to 15' of experimental harassment prior to capture. Dashed lines mark CORT baseline levels (average of the "naïve control" group). All values in means ± 1SE.

(iii) acute, heavy predation risk

On San Cristobal, we witnessed acute heavy predation in a population suffering frequent attacks from dogs. We observed numerous corpses and bite marks on the tails of live individuals. In contrast to the other sites, this heavy acute predation threat resulted in significant CORT increases to the threat of experimental chasing by a human even in naïve chased animals (t = -3.678, df = 21, p < 0.001). Furthermore, CORT responses to restraint were highest at this site and naïve iguanas had the longest FIDs on this island.

We supplemented our results of adult males by replicating the comparisons of CORT concentrations between naïve controls and chased groups with animals between 2 and 4 years of age (Fig. 3). Similar to adult males, young iguanas did not respond to experimental chasing with a CORT increase (Caamaño t = -1.095, df = 23, p = 0.285, Punta Nuñez t = -0231, df = 18, p = 0.820). However, restraint caused a significant CORT increase at both sites (Caamaño t = -6.137, df = 18, p < 0.001, and Punta Nuñez t = -7.408, df = 21, p < 0.001).

5.5 DISCUSSION

Combining the results from three different sites reveals a coherent pattern: Predator-naïve marine iguanas elicit flight very late and fail to mount a stress response during sustained chasing - they are predator-naïve in behaviour and in endocrine control systems, congruently. When living with predators on Punta Nuñez, chased animals still did not activate their HPA axis, even though a recent capture experience primed marine iguanas to human approach and elicited a CORT response, indistinguishable in strength to the response to restraint. At the same time FIDs were significantly higher than in naïve controls. Marine iguanas at the predator-ridden site on San Cristobal elicited a CORT response to chasing even without a prior bad experience with humans.

Our results demonstrate for the first time that low wariness in a naïve insular organism is accompanied by the failure to activate the HPA-axis, suggesting deficient predator recognition. A one-time experience with a novel predation attempt (capture), however, was enough to increase FID and, in combination with predator presence, to activate a CORT response to chasing to a similar degree as restraint. Living among predators seems to prime the HPA system, which then allows a rapid change from being non-responsive to being responsive to a novel stimulus (the human experimenter). Because the HPA system is important for surviving common metabolic stressors in marine iguanas (Romero & Wikelski 2001), marine iguanas appear to retain classic HPA function even through long evolutionary periods of time without predators. The HPA system can quickly regain its activity if predation resumes since at San Cristobal, where animals were likely to have experienced recent (failed) predation attempts, the HPA system was activated even after an unknown human stimulus.

Most relevant in terms of fitness, however, is the question of whether the described FID changes will suffice to survive and thrive in the face of introduced predators. The answer is most likely negative. Although marine iguanas increased FID after a one-time experience, the magnitude of change was small. The authors could recapture experienced animals with a noose on a 3 m pole repeatedly up to six times, as has been done for a previous experiment (Berger et al. 2005). Accordingly, introduced cats and dogs are known to cause large scale depredations among marine iguana populations (Kruuk & Snell 1981; Laurie 1983). On San Cristobal near the town Puerto Baquerizo Moreno, dogs drove the local study population to near extinction within a short time.

Because changes in FID were insufficient in absolute terms, it becomes counterintuitive that a behavioural trait is more refractory than a corresponding physiological control system. One possible interpretation is that experience with predators adjusted CORT responses within the reaction norm for other, metabolic, stressors. In contrast, no familiar equivalents to a predator exist in a marine iguana's life history that would stimulate as high a flight response as is necessary to escape a mammalian predator. In other words, the reaction norm for CORT responses is broad enough to encompass the necessary adjustments to a novel predator, but the reaction norm for behavioural responses is too narrow to encompass an effective response. In support of this idea, the magnitude of FID differences between naïve and previously caught animals is comparable to the magnitude of differences based on age and sex (Berger et al. in prep.). Our results strongly suggest that the estimated 5-15 million years of predator release (Rassmann 1997) narrowed the reaction norm for FID too far for marine iguanas to adjust their behaviour fast enough to an unknown stimulus in order to minimise predation by introduced mammals, which have been present for no longer than 150 years. We suggest a similar pattern in other insular vertebrates with long periods of evolutionary isolation from predators: high phenotypic plasticity of the physiological stress response to novel stressors but restricted plasticity in flight behaviour. There is a high need for further studies on the generality of limited reaction norms in predator-naïve organisms. This will improve our general knowledge on the interactions between wariness and its physiological control mechanisms; and it will help wildlife managers (i) when predicting the impact of introduced predators on natural island populations, and (ii) when selectively modifying behavioural responses of bred animals prior to re-introductions (Beck et al. 1994; Maloney & MacLean 1995; Griffin et al. 2000).

5.6 ACKNOWLEDGMENTS

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Curriculum Vitae

Silke Berger

Address:	Steinbruchweg 15
	74564 Crailsheim
Phone:	+49 7951 22620
Date of birth:	15 March 1973
Nationality:	German
Languages:	German, English, French

EDUCATION

Oct. 1996 - Feb. 2002	Diplom (≈MSc) in Biology, Julius-Maximilians-University of Würzburg, Germany, in "Tropical Biology and Ecology", "Botany", and "Pharmaceutical Biology".
	Diplom thesis "Why do starling males carry fresh herbs into their nests?" at the Max-Planck-Institute for Behavioral Physiology, Research Centre for Ornithology, Andechs, Germany.
Sep. 1993 - Sep. 1996	Professional training as a bank assistant.
June 1993	German Abitur (final high-school degree), Economy Gymnasium Crailsheim.

PH.D. THESIS

Since August 2002: "Influence of introduced predators and natural stressors on escape behavior and endocrine mechanisms in an island species, the Galápagos marine iguana *(Amblyrhynchus cristatus)*."

TEMPORARY WORK CONTRACT

März 2002 – August 2002: Scientific assistant at the Max-Planck-Institute for Behavioral Physiology, Research Centre for Ornithology, Andechs, Germany.
Project: "Nest building behavior and breeding biology in European starlings *(Sturnus vulgaris)*".

RELATED WORK ACTIVITY

2000	Practical course at the bird station of Helgoland, Wilhelmshaven (Germany).
1999	Intensive summer field course in Ivory Coast (West Africa) on primary and secondary seed dispersal.

FINANCIAL SUPPORT

Mai 2003 – Aug. 2005	Gottlieb Daimler- und Karl Benz foundation, Ph.D. fellowship.
2002	Arthur v. Gwinner foundation for scientific research expeditions, grant in aid for equipment and travel expenses (Galápagos project).

PUBLICATIONS

2006	Rödl, T., Berger S., Romero, L. M. & Wikelski, M (2006) Tameness and stress physiology in a predator-naïve island species confronted with novel predation threat. Proceedings of the Royal Society B: Biological Sciences. Online.
2005	Berger, S., Martin II, L. B., Wikelski, M., Romero, L. M., Kalko, E. K. V., Vitousek, M. N. & Rödl, T. (2005) Corticosterone suppresses immune activity in territorial Galápagos marine iguanas during reproduction. Hormones & Behavior. 47: 419-429.
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2004   Berger, S., Wikelski M., Kalko, E. K. V. & Rödl, T. (2004) Immune activity in male marine iguanas *(Amblyrhynchus cristatus)* during reproduction. Second European Conference on Behavioural Biology (ECBB), Groningen, Netherlands, August 2004 (poster).

2004   Rödl, T., Berger, S. & Wikelski, M. (2004) Trouble in paradise – about stress, Galápagos iguanas, and introduced predators. Alexander v. Humboldt Association of America, 5th Bi-annual national meeting, Washington D.C., June 2004 (poster).

2003   Berger, S., Martin II, L. B., Rödl, T., Vitousek, M. N., Romero, L. M., Wikelski, M. & Kalko, E. K. V (2003). Immune function, steroid hormones, and male reproductive strategies in the Galápagos marine iguana (*Amblyrhynchus cristatus*). Animal Behavior Society annual meeting, Boise, ID, USA, July 2003 (poster).

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2003   Mitchell, M. A., Nevarez, J., Dae-Young, K., Rödl, T., Berger, S. & Wikelski, M. (2003) Evaluating the long-term health effects of an unnatural oil spill on marine iguanas (*Amblyrhynchus cristatus*). Association of reptilian and amphibian veterinarians annual conference, Minneapolis, MN, Oct. 2003 (talk).

2003   Gwinner, H. & Berger, S. (2003) Parasite defence in birds. The role of volatiles. Proceedings of the 23 th International IOC. Acta Zoologica Sinica. (written review).

2001   Gwinner, H. & Berger, S. (2001) Aromatic herbs in starling nests. XXVII. International Ethological Conference in Tübingen, Germany (poster).

2000   Berger, S., Kunz, B. K. & Linsenmair, K. E. (2000) Observations of frugivorous birds and mammals in the tree *Lannea barteri* (Anacardiaceae). 13th Conference of the German Society of Tropical Ecology in Würzburg, Germany (poster).

Eidesstattliche Erklärung

Hiermit erkläre ich, die vorliegende Dissertationsarbeit selbständig angefertigt und keine anderen als die in der Arbeit aufgeführten Hilfsmittel verwendet zu haben. Wörtlich oder inhaltlich übernommene Stellen wurden als solche gekennzeichnet.

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